October 8, 2001

By Mail
Christine Todd Whitman, Administrator
US EPA
PO Box 1473
Merrifield, VA 22116

Attn: Chemical Right-to-Know Program – Test Plan Submission from HERTG
Registration Number

Dear Administrator Whitman:

The American Chemistry Council Petroleum Additives Panel (Panel) Health, Environmental, and Regulatory Task Group (HERTG) submits for review and public comment its test plan report, as well as related robust summaries, for the *"alkaryl sulfonate"* category of chemicals under the Environmental Protection Agency's High Production Volume (HPV) Chemical Challenge Program. The HERTG understands that there will be a 120-day review period for the test plan report and that all comments generated by or provided to EPA will be forwarded to the HERTG for consideration.

The alkaryl sulfonates in this category, which are used as petroleum lubricant additives, are characterized by having structural similarities and limited reactivity, low biological activity, and very low water solubility. Based upon the data reviewed in the attached report, the HERTG concludes that the physicochemical and toxicological properties of the proposed alkaryl sulfonate category members are similar and follow a regular pattern as a result of structural similarity. Thus, HERTG believes these twelve chemicals meet the EPA definition of a chemical category and will test them in accordance with the test plan summarized in the attached report. The twelve chemicals in the alkaryl sulfonate category are as follows:

- sulfonic acids, petroleum, calcium salts - (CAS # 61789-86-4, referred to in this report as petroleum derived calcium salt)
- sulfonic acids, petroleum, barium salts - (CAS # 61790-48-5, referred to in this report as petroleum derived barium salt)
- sulfonic acids, petroleum, sodium salts - (CAS # 68608-26-4, referred to in this report as petroleum derived sodium salt)
- sulfonic acids, petroleum, calcium salts, overbased - (CAS # 68783-96-0, referred to in this report as petroleum derived calcium salt, overbased)
- benzenesulfonic acid, mono-C16-C24 alkyl derivatives, calcium salts- (CAS # 70024-69-0, referred to in this report as C16-C24 alkaryl calcium salt derivative)
- benzenesulfonic acid, mono-C15-C30 branched alkyl and di-C11-C13 branched and linear alkyl derivatives, calcium salts, overbased - (CAS # 71486-79-8, referred to in this report as mixed mono-C15-C30 and di-C11-C13 alkaryl calcium salt, overbased derivative)
- benzenesulfonic acid, mono-C15-C30 branched alkyl and di-C11-C13 branched and linear alkyl derivatives - (CAS # 71549-79-6, referred to in this report as mixed mono-C15-C30 and di-C11-C13 alkaryl derivative)

- benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts - (CAS # 71786-47-5, referred to in this report as alkaryl magnesium salt derivative)
- benzenesulfonic acid, C15-C30 alkyl derivatives, sodium salts - (CAS # 78330-12-8, referred to in this report as C15-C30 alkaryl sodium salt derivative)
- benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives, calcium salts - (CAS # 115733-09-0, referred to in this report as C14-C24 alkaryl calcium salt derivative)
- benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives, calcium salts, overbased - (CAS # 115733-10-3, referred to in this report as C14-C24 alkaryl calcium salt, overbased derivative)
- benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives - (CAS # 115829-36-2, referred to in this report as C14-C24 alkaryl derivative)

Briefly, the test plan for the HERTG alkaryl sulfonate category includes the following tests and computer modeling:

- Water solubility – Petroleum derived sodium salt (CAS #68608-26-4), C15-C30 alkaryl sodium salt (CAS #78330-12-8), and C14-C24 alkaryl calcium salt derivative (CAS # 115733-09-0) will be tested.
- Biodegradability – C15-C30 alkaryl sodium salt (CAS # 78330-12-8) will be tested.
- Photodegradation (atmospheric oxidation) modeling – Data will be developed using the AOP model in EPIWIN.
- Fugacity modeling – Environmental partitioning data will be calculated using a Mackay Level I equilibrium partitioning model.
- Acute fish toxicity - Limit tests will be conducted on petroleum derived barium salt (CAS # 61790-48-5), petroleum derived sodium salt (CAS # 68608-26-4), petroleum derived calcium salt, overbased (CAS # 68783-96-0), and C14-C24 alkaryl calcium salt derivative (CAS # 115733-09-0).
- Acute invertebrate toxicity - Limit tests will be conducted on petroleum derived sodium salt (CAS # 68608-26-4) and petroleum derived calcium salt, overbased (CAS # 68783-96-0).
- Alga toxicity - Limit tests will be conducted on petroleum derived sodium salt (CAS # 68608-26-4) and petroleum derived calcium salt, overbased (CAS # 68783-96-0).
- Repeated-dose toxicity - C14-C24 alkaryl calcium salt derivative (CAS # 115733-09-0) will be tested in a 28-day dose-range finding study for the reproductive/developmental toxicity study.
- Reproductive/developmental toxicity - C14-C24 alkaryl calcium salt derivative (CAS # 115733-09-0) will be tested in a one-generation study.

As HERTG developed this test plan, HERTG considered carefully and tried to limit how many animals might be required for tests included in the proposed plan and conditions to which the animals might be exposed. As noted above, a minimal amount of animal testing is proposed and, for those tests, HERTG believes the currently available scientific evidence suggests no significant toxicity will be demonstrated. As a result, HERTG believes that the concerns of some non-governmental organizations about animal welfare have been fully considered and that use of animals in this proposed test plan has been minimized.

Included in this package is a computer diskette that contains electronic copies of the HERTG's test plan report and accompanying robust summaries.

Thank you in advance for your attention to this matter. If you have any questions regarding the test plan report or the robust summaries, or HERTG's activities associated with the Challenge Program, please me at 703-741-5607 (telephone), 703-741-6091 (telefax) or Sarah_loftus@americanchemistry.com (e-mail).

Sincerely,

Sarah C. Loftus
HERTG Technical Contact, US HPV Challenge

cc: HERTG members

AR201-13206A

HIGH PRODUCTION VOLUME (HPV)

CHALLENGE PROGRAM

TEST PLAN

For

PETROLEUM ADDITIVE ALKARYL SULFONATE CATEGORY

Prepared by

The American Chemistry Council
Petroleum Additives Panel
Health, Environmental, and Regulatory Task Group

October 2001

List of Member Companies in the Health, Environmental, and Regulatory Task Group

The Health, Environmental, and Regulatory Task Group (HERTG) of the American Chemistry Council Petroleum Additives Panel includes the following member companies:

B.P. plc

Chevron Oronite Company, LLC

Crompton Corporation

Ethyl Corporation

ExxonMobil Chemical Company

Ferro Corporation

Infineum

The Lubrizol Corporation

Rhein Chemie Corporation

Rhodia. Inc.

EXECUTIVE SUMMARY

The American Chemistry Council Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its member companies, hereby submit for review and public comment its test plan for the *"petroleum additive alkaryl sulfonate"* category of chemicals under the Environmental Protection Agency's High Production Volume (HPV) Challenge Program. This report should be read in its entirety in order to obtain an understanding of the category and proposed testing.

Alkaryl Sulfonate Category. Based on several factors specified in EPA's guidance document on "Development of Chemical Categories in the HPV Challenge Program," in which use of chemical categories is encouraged, the following twelve closely related chemicals constitute a chemical category:

- sulfonic acids, petroleum, calcium salts - (CAS # 61789-86-4, referred to in this report as petroleum derived calcium salt)
- sulfonic acids, petroleum, barium salts - (CAS # 61790-48-5, referred to in this report as petroleum derived barium salt)
- sulfonic acids, petroleum, sodium salts - (CAS # 68608-26-4, referred to in this report as petroleum derived sodium salt)
- sulfonic acids, petroleum, calcium salts, overbased - (CAS # 68783-96-0, referred to in this report as petroleum derived calcium salt, overbased)
- benzenesulfonic acid, mono-C16-C24 alkyl derivatives, calcium salts - (CAS # 70024-69-0, referred to in this report as C 16-C24 alkaryl calcium salt derivative)
- benzenesulfonic acid, mono-C 15-C30 branched alkyl and di-C 11 -C 13 branched and linear alkyl derivatives, calcium salts, overbased - (CAS # 71486-79-8, referred to in this report as mixed mono-C 1 5-C30 and di-C 11 -C 13 alkaryl calcium salt, overbased derivative)
- benzenesulfonic acid, mono-C 15-C30 branched alkyl and di-C 11 -C 13 branched and linear alkyl derivatives - (CAS # 71549-79-6, referred to in this report as mixed mono-C 15X30 and di-C 11 -C 13 alkaryl derivative)
- benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts - (CAS # 71786-47-5, referred to in this report as alkaryl magnesium salt derivative)
- benzenesulfonic acid, Cl 5-C30 alkyl derivatives, sodium salts - (CAS # 78330- 12-8, referred to in this report as C15-C30 alkaryl sodium salt derivative)
- benzenesulfonic acid, C 14-C24 branched and linear alkyl derivatives, calcium salts - (CAS # 115733-09-0, referred to in this report as C14-C24 alkaryl calcium salt derivative)
- benzenesulfonic acid, C 14-C24 branched and linear alkyl derivatives, calcium salts, overbased - (CAS # 115733-10-3, referred to in this report as C14-C24 alkaryl calcium salt, overbased derivative)
- benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives - (CAS # 115829-36-2, referred to in this report as C14-C24 alkaryl derivative)

Structural Similarity. A key factor supporting the classification of these chemicals as a category is their structural similarity. All substances in this category consist of a benzene ring with a sulfonic acid substituent group and one or more long-chain alkyl substituent groups that vary in length and extent of branching. Most of these substances have been neutralized by an alkali metal base to form the corresponding alkali metal salt.

Similarity of Physicochemical Properties. The similarity of the *physicochemical properties* of these substances parallels their structural similarity. All are dark colored viscous liquids intended for use as components in finished lubricating oils. The use of these substances in finished lubricants requires that they be stable under high temperatures (>100°C). Their low volatility is due to their low vapor pressure, high viscosity, and relatively high molecular weights. The existing information for these substances indicates that they have low water solubility. However, additional water solubility data will be collected.

Fate and Transport Characteristics. Members of this category have been shown to be poorly biodegradable. However, additional biodegradation testing will be conducted to determine whether there is potential for a higher degree of biodegradability for members of this category that have linear alkyl groups. Since the members of this category have low water solubility, hydrolysis testing is technically unfeasible. Furthermore, members of the category are resistant to hydrolysis because they lack hydrolyzable moieties, This makes hydrolysis modeling unnecessary. Photodegradation is not expected to cause significant physical degradation of petroleum additive alkaryl sulfonates. However, computer-modeled data will be developed to adequately characterize the potential atmospheric oxidation potential for members of this category. Although these substances are not expected to partition to water or air if released into the environment due to their low water solubility and low vapor pressure, computer-modeled environmental partitioning data will be calculated on the members of this category.

Toxicological Similarity. Review of existing published and unpublished test data for petroleum additive alkaryl sulfonates shows the *aquatic and mammalian toxicity* among the twelve substances within this category are similar and are of a low concern.

Aquatic Toxicology. Data on acute fish toxicity, acute invertebrate toxicity, and alga toxicity were reviewed, and the findings indicate little to no toxicity to fish, aquatic invertebrates, and alga when appropriate test methods are used. However, additional tests will be conducted so that this category may be adequately characterized for aquatic toxicity.

Mammalian Toxicology - Acute. Data on acute mammalian toxicity were reviewed, and the findings indicate a low concern for acute toxicity. Data are available for most members of the category indicating that the category has been well tested for acute mammalian effects. Therefore, no additional acute mammalian toxicity testing is necessary.

Mammalian Toxicology - Mutagenicity. Data from bacteria 1 reverse mutation assays and *in vitro* and *in vivo* chromosome aberration studies were reviewed, and the findings indicate a low concern for mutagenicity. Data are available for several members of the category or structural analogs, and these data can be bridged to the other members of the category. Therefore, the

category has been adequately tested for mutagenicity, and no additional mutagenicity testing is necessary.

Mammalian Toxicology - Subchronic Toxicity. Data from repeated-dose toxicity studies were reviewed. Minimal signs of toxicity were observed following repeated oral exposure. Adverse effects at the site of contact were observed following repeated dermal exposure (injury to the skin) and repeated inhalation (injury to the lungs). These findings can be bridged to the remaining members of the category. However, an additional repeated-dose toxicity study will be conducted as a dosssetting study for the reproductive toxicity described below.

Mammalian Toxicology - Reproductive and Developmental Toxicity. There are no published or unpublished reproductive/developmental studies for members of the petroleum additive alkaryl sulfonate category. A one-generation reproductive toxicity test will be conducted to provide data that can be bridged to the remainder of the category.

Conclusion. Based upon the data reviewed in the report, the physicochemical and toxicological properties of the proposed petroleum additive alkaryl sulfonate category members are similar and follow a regular pattern as a result of that structural similarity. Therefore, the EPA definition of a chemical category has been met, and the twelve chemicals that constitute the petroleum additive alkaryl sulfonate category will be tested in accordance with the test plan summarized below.

Test Plan. The test plan for the petroleum additive alkaryl sulfonate category includes the following tests and computer modeling:

- Water solubility – Petroleum derived calcium salt (CAS # 61789-86-4), petroleum derived barium salt (CAS # 61790-48-5), petroleum derived sodium salt (CAS # 68608-26-4), C15-C30 alkaryl sodium salt (CAS # 78330-12-8), and C14-C24 alkaryl calcium salt derivative (CAS # 115733-09-O) will be tested.
- Biodegradability – C 15-C30 alkaryl sodium salt (CAS # 78330-12-8) will be tested.
- Photodegradation (atmospheric oxidation) modeling – Data will be developed using the AOP model in EPIWIN.
- Fugacity modeling – Environmental partitioning data will be calculated using a Mackay Level I equilibrium partitioning model.
- Acute fish toxicity - Limit tests will be conducted on petroleum derived barium salt (CAS # 6 1790-48-Q petroleum derived sodium salt (CAS # 68608-26-4), petroleum derived calcium salt (CAS # 61789-86-4), and C14-C24 alkaryl calcium salt derivative (CAS # 115733-09-o).
- Acute invertebrate toxicity - Limit tests will be conducted on petroleum derived sodium salt (CAS # 68608-26-4) and petroleum derived calcium salt (CAS # 61789-86-4).
- Alga toxicity - Limit tests will be conducted on petroleum derived sodium salt (CAS # 68608-26-4) and petroleum derived calcium salt (CAS # 61789-86-4).
- Repeated-dose toxicity - C14-C24 alkaryl calcium salt derivative (CAS # 115733-09-o) will be tested in a 28-day dose-range finding study for the reproductive/developmental toxicity study.
- Reproductive/developmental toxicity - C 14-C24 alkaryl calcium salt derivative (CAS # 115733-09-o) will be tested in a one-generation study.

As this test plan was developed, careful consideration was given to the number of animals that would be required for tests included in the proposed plan and conditions to which the animals might be exposed. In consideration of the concerns of some non governmental organizations about animal welfare, the use of animals in this proposed test plan has been minimized.

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1.0 INTRODUCTION

In March 1999, the American Chemistry Council (formerly the Chemical Manufacturers Association) Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its participating member companies committed to address data needs for certain chemicals listed under the Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program. This test plan follows up on that commitment.

Specifically, this test plan sets forth how the HERTG intends to address testing information for the twelve substances listed in Table 1 and represented structurally in Table 2. These twelve substances include:

- sulfonic acids, petroleum, calcium salts - (CAS # 61789-86-4, referred to in this report as petroleum derived calcium salt)
- sulfonic acids, petroleum, barium salts - (CAS # 61790-48-5, referred to in this report as petroleum derived barium salt)
- sulfonic acids, petroleum, sodium salts - (CAS # 68608-26-4, referred to in this report as petroleum derived sodium salt)
- sulfonic acids, petroleum, calcium salts, overbased - (CAS # 68783-96-0, referred to in this report as petroleum derived calcium salt, overbased)
- benzenesulfonic acid, mono-C16-C24 alkyl derivatives, calcium salts- (CAS # 70024-69-0, referred to in this report as C16-C24 alkaryl calcium salt derivative)
- benzenesulfonic acid, mono-C 15-C30 branched alkyl and di-C 11 -Cl 3 branched and linear alkyl derivatives, calcium salts, overbased - (CAS # 71486-79-8, referred to in this report as mixed mono-C 15-C30 and di-Cl 1 -Cl3 alkaryl calcium salt, overbased derivative)
- benzenesulfonic acid, mono-C 15-C30 branched alkyl and di-C 11 -C 13 branched and linear alkyl derivatives - (CAS # 71549-79-6, referred to in this report as mixed mono-C15-C30 and di-C1 1-C13 alkaryl derivative)
- benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts - (CAS # 71786-47-5, referred to in this report as alkaryl magnesium salt derivative)
- benzenesulfonic acid, C15-C30 alkyl derivatives, sodium salts - (CAS # 78330-12-8, referred to in this report as C15-C30 alkaryl sodium salt derivative)
- benzenesulfonic acid, C 14-C24 branched and linear alkyl derivatives, calcium salts - (CAS # 115733-09-0, referred to in this report as C14-C24 alkaryl calcium salt derivative)
- benzenesulfonic acid, C 14-C24 branched and linear alkyl derivatives, calcium salts, overbased - (CAS # 115733-10-3, referred to in this report as C14-C24 alkaryl calcium salt, overbased derivative)
- benzenesulfonic acid, C 14-C24 branched and linear alkyl derivatives - (CAS # 115829-36-2, referred to in this report as C14-C24 alkaryl derivative)

An analysis of the available data on these chemicals supports the designation of the petroleum additive alkaryl sulfonates as a "chemical category" as provided in the EPA guidance document entitled, "Development of Chemical Categories in the HPV

Challenge Program." This document provides the basis for that determination, indicates the findings of the data review process, and sets forth a proposed test plan to satisfy parts of the required test battery for endpoints without data that would be considered adequate under the program.

EPA guidance on the HPV Challenge Program indicates that the primary purpose of the program is to encourage "the chemical industry . , . to voluntarily compile a Screening Information Data Set (SIDS) on all chemicals on the US HPV list." (EPA, "Development of Chemical Categories in the HPV Challenge Program," p. 1) At the same time, EPA recognizes that the "large number of chemicals to be tested [about 2800 HPV chemicals] makes it important to reduce the number of tests to be conducted, where *this is scientifically justifiable.*" (Id., p. 1) [emphasis added]. The next part of the guidance explains where this would be scientifically justifiable:

> One approach is to test closely related chemicals as a group, or category, rather than test them as individual chemicals. In the category approach, not every *chemical needs to be tested for every SIDS endpoint.* However, *the test data finally compiled* for the category must prove adequate to support a screening level hazard-assessment of the category and its members. That is, the *final data set* must allow one to estimate the hazard for the untested endpoints, *ideally* by interpolation between and among the category members. In certain cases, where toxicity is low and no upward trend is expected, extrapolation to the higher category members may be acceptable. *(Id.,* p. 1) [emphasis added].

EPA guidance goes on to state, "The use of categories is encouraged in the Challenge Program and will have a number of benefits." *(Id.,* p. 1) Among the benefits identified in the guidance for the use of categories are "a reduction in testing will result in fewer animals used to test a category of chemicals as opposed to doing each test on each individual chemical," and "there will be . . . economic savings since less testing may be needed for chemicals considered as a category." *(Id.,* p. 1) That guidance also states that categories "accomplish the goal of the Challenge Program – to obtain screening level hazard information – through the strategic application of testing to the category." *(Id,* p. 2)

A similarly stated intent "to reduce the number of tests to be conducted, *where this is scientifically justifiable*" was articulated by the Agency in its draft guidance document titled, "The Use of Structure Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program." [emphasis added].

The EPA "Chemical Categories" guidance sets forth a definition of what constitutes a "chemical category, for the purposes of the Challenge Program." Specifically, that definition states that a chemical category under the HPV Challenge Program "is a group of chemicals whose physicochemical and toxicological properties *are likely to* be similar *or* follow a regular pattern as a result of structural similarity." *(Op. Cit.,* p. 2) [emphasis added].

According to the guidance, what is important is that the "structural similarities [among members of the group] *may* create a predictable pattern in any or all of the following parameters: physicochemical properties, environmental fate and effects, and human health effects." (Id., p. 2) [emphasis added]. Thus, it is not necessary for the chemicals in a category to be similar in all respects. Nor must there be conclusive proof that the chemicals in the postulated category will behave identically across all relevant parameters. All that is required for an acceptable category under the HPV Challenge Program is that there be a *likelihood* of similarity of physicochemical and toxicological properties or a *likelihood* that the chemicals will in some pertinent respect follow a regular pattern as a result of their structural similarity.

In identifying the petroleum additive alkaryl sulfonate category, the six-step process set out in the EPA guidance on category development was followed. As the information below indicates, the petroleum additive alkaryl sulfonate category of chemicals clearly satisfies the standards established in that guidance for use of a chemical category:

Step 1: group structurally similar chemicals into a putative category

Step 2: gather relevant published and unpublished literature for each member of the category

Step 3: evaluate the compiled data for adequacy in accordance with the EPA guidance documentation

Step 4: construct matrices of SIDS endpoints versus category members arranged so as to indicate the structural progression of the category (in this case, by increasing alkyl side chain length in Tables 3-9)

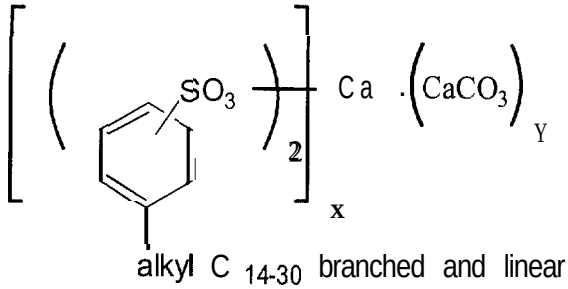
Step 5: evaluate the data to determine whether there is a correlation between category members for each SIDS endpoint

Step 6: make available to EPA, and to the public for review, this test plan including the foregoing category definition and rationale and the following data assessment with the proposed testing scheme for the petroleum additive alkaryl sulfonates.

2.0 CHEMICAL DESCRIPTOIN OF ALKYL SULFIDE CATEGORY

2.1 DESCRIPTION

Alkaryl sulfonates consist of a benzene ring with a sulfonic acid substituent group and one or more long-chain alkyl substituent groups. The alkyl groups are saturated hydrocarbon chains that vary in length and extent of branching. A basic metal may neutralize the acid group, and calcium is typically used. A general structure is shown below.



As a general class, these substances are commonly used as detergents and surface-active agents by many industries. However, subclasses of alkaryl sulfonates exist with somewhat different physical and chemical properties based on the performance requirements of the various industries. For example, the consumer detergent industry tends to use alkaryl sulfonates with alkyl groups of 12 carbons or less to support miscibility in water.

The alkaryl sulfonates that are the subject of this test plan are used as petroleum additives in petroleum base stocks. The chemical names and CAS numbers for the members of the petroleum additive alkaryl sulfonate category are presented in Table 1. The chemical structures for the members of the category are presented in Table 2. These substances are prepared by sulfonation of either synthetic alkylbenzene substrates or naturally occurring alkylaromatic-rich fractions of heavy lubricating oil base stocks derived from petroleum streams. The alkyl substituent group may vary in number (e.g., mono- or dialkyl), position (e.g., predominantly meta or para to the sulfonic acid position), chain length (e.g., Cl4 to C30) or in the degree of branching. The distribution of the alkyl chain lengths for several members of the category are presented in Figure 1. Branched and linear alkyl groups of 20 or more carbons are used to enhance oil solubility. Although branched alkyl groups are generally presumed to be more water-soluble than straight chains, petroleum additive alkaryl sulfonates have such low water solubility that the degree of branching does not affect their solubility and performance in petroleum base stocks.

The petroleum additive alkaryl sulfonates are manufactured in petroleum base stocks, and thus the substrates are never isolated. The sulfonic acid substituent group can be neutralized by alkali metal bases to form the corresponding alkali metal salt. For the members of the petroleum additive alkaryl sulfonate category, the sulfonic acid substituent group may be present as the free acid or as a salt of sodium, calcium, magnesium or barium. The salts can also be complexed ("overbased") with an excess of metal carbonate. The overbased products are produced in the presence of the alkaryl sulfonic acid salt (soap) by adding excess metal hydroxide and carbon dioxide. The over basing reaction forms the metal carbonate which exists in the lubricating oil diluent as a reverse micelle (i.e., the metal cabonate is in the center of the micelle with the alkaryl sulfonic acid salt [soap] surrounding the carbonate). Figure 2 shows the general structure of a petroleum additive alkaryl sulfonate reverse micelle. The ratio of metal carbonate to soap can range from a low of 6: 1 to a high of 30: 1. As the ratio increases, the alkaryl

sulfonic acid salt (soap) content is diluted. Thus, the overbased members of the category are considered more dilute analogs of the category members that are not overbased.

2.2 PHYSICOCHEMICAL PROPERTIES

The physicochemical properties of the members of the petroleum additive alkaryl sulfonate category are presented in Table 3. They are all dark colored viscous liquids at ambient temperature. The similarities in the other physicochemical properties of these substances, which are described below, are explained by similarities in their chemical structure and processing and provide justification of this group of chemicals as a category within the HPV Challenge Program.

2.1.1 Molecular Weight and Alkyl Side Chain Length

The members of the category range in molecular weight from 354 to 1194 daltons. However, these substances will dissociate to some degree in aquatic environments and biological systems, and it is better to characterize the members by their equivalent weight to evaluate their toxicity. The equivalent weight is the weight of one alkylbenzene sulfonic acid plus the weight of the alkali metal.

Two structural variables in the category influence the variability in equivalent weight of the category members: the alkali metal and the alkyl side chain length. The latter also has an influence on water solubility and the ability of the member of the category to cross biological membranes, which in turn influences bioavailability. The carbon chain length range for each member of the category is presented in Table 3, and the fractional distribution of each carbon number is illustrated in Figure 1 for several members of the category. Due to the influence of carbon chain length on bioavailability, the members of the category are arrayed in order of increasing carbon chain length in Tables 3-9.

2.1.2 Specific Gravity

Available specific gravity data are presented in Table 3. The specific gravity of the overbased members of the category is approximately 1.1. The specific gravity of the category members that are not overbased is slightly less than 1.0.

2.1.3 Viscosity

Available viscosity data are presented in Table 3. As manufactured in petroleum base stocks, the viscosity of the members of the petroleum additive alkaryl sulfonate category is approximately 200 cSt @ 100°C (Table 3) and 1500 cSt @ 40°C.

2.1.4 Melting Point

The high viscosity of the members of the category makes it technically unfeasible to determine their melting point. However, modeling data indicates that the melting point of the "de-oiled" substances ranges from 208°C to 350°C (Table 3).

2.1.5 Boiling Point

The use of these substances in finished lubricants requires that they be thermally and chemically stable under high temperatures (>1 00°C). Typically, the petroleum base stocks in these substances boil at temperatures above 300°C. Modeling data indicates that the boiling point of the "de-oiled" substances ranges from 506°C to 936°C (Table 3).

2.1.6 Vapor Pressure

Since "de-oiled" petroleum additive alkaryl sulfonates are solid, their vapor pressure can be estimated from the vapor pressure of the petroleum base stocks in which they are manufactured. Typically, these base oil stocks have low vapor pressure, $< 10^{-10}$ Pa @ 25°C (Table 3). Thus, the low volatility of the members of the petroleum additive alkaryl sulfonates is due to their low vapor pressure, high viscosity, and high relative molecular weights.

2.1.7 Water Solubility and Octanol-Water Partition Coefficients

Data for mixed mono-C1 5-C30 and di-Cl 1-C13 alkaryl derivative (CAS # 71549-79-6) and a C20-C24 alkaryl calcium salt derivative (no CAS #) analog of C16-C24 alkaryl calcium salt derivative (CAS # 70024-69-o) indicate that these substances have low water solubility, < lppm (Table 3). The log of the octanol-water partition coefficients of these substances is greater than 6.0 (Table 3).

The low water solubility of these two substances is consistent with the presence of the long hydrocarbon side chain. However, additional water solubility data will be determined for one substance that contains a shorter alkyl side chain, C 14-C24 alkaryl calcium salt derivative (CAS # 115733-09-0), and two substances that are sodium salts, petroleum derived sodium salt (CAS # 6860%26-4), C15-C30 alkaryl sodium salt derivative (CAS # 78330-12-g). Water solubility will also be determined on two additional substances to support their acute aquatic toxicity evaluation, petroleum derived calcium salt (CAS # 6 1789-86-4) and petroleum derived barium salt (CAS 6 1790-48-5).

3.0 USES OF PETROLEUM ADDITIVE ALKARYL SULFONATES

Petroleum additive alkaryl sulfonates are used to formulate finished lubricating oils including all types of automotive and diesel engine crankcase oils, air and water-cooled two-cycle engine oils, industrial oils, hydraulic fluids, gear oils, and metal working lubricating oils. They are used as high temperature detergents to reduce deposits on

pistons, engine crankcases, and hydraulic equipment parts and as rust inhibitors during industrial oil use. Petroleum additive alkaryl sulfonates are generally sold to finished oil blenders in additive packages, where the concentration ranges from 1 to 50 wt.%. These additive packages are then blended into finished oils where the typical concentration of alkaryl sulfonate ranges from 0.1 to 10 wt.% in the finished oil.

Petroleum additive alkaryl sulfonates are manufactured and blended into additive packages at plants owned by members of the HERTG. Finished lubricants are blended at facilities owned by our customers. Additive packages are shipped to customers in bulk in ships, isocontainers, railroad tank cars, tank trucks or in 55-gallon steel drums. The bulk additive packages are stored in bulk storage tanks at the customer blending sites. Finished oils are blended by pumping the lubricating oil blend stocks and the additive package from their storage tanks through computer controlled valves that meter the precise delivery of the components into a blending tank. After blending, the finished lubricant products are sold in bulk and shipped in tank trucks to large industrial users, such as manufacturing facilities and facilities that service truck fleets and passenger motor vehicles. Finished lubricants are also packaged into 55-gallon drums, 5-gallon pails, and one-gallon and one-quart containers for sale to smaller industrial users. Sales of lubricants in one-gallon and one-quart containers to consumers at service stations or retail specialty stores also occur.

Based on these uses, the potentially exposed populations include (1) workers involved in the manufacture of alkaryl sulfonates, blending them into additive packages, and blending the additive packages into finished lubricants; (2) quality assurance workers who sample and analyze these products to ensure that they meet specifications; (3) workers involved in the transfer and transport of alkaryl sulfonates, additive packages or finished lubricants that contain them; (4) mechanics who may come into contact with both fresh and used lubricants while working on engines or equipment; (5) gasoline station attendants and consumers who may periodically add lubricating oil to automotive crankcases; and (6) consumers who may change their own automotive engine oil. The most likely route of exposure for these substances is skin and eye contact. Manufacturing, quality assurance, and transportation workers will likely have access to engineering controls and wear protective clothing to eliminate exposure. Mechanics wear protective clothing, but often work without gloves or eye protection. Gasoline station attendants and consumers often work without gloves or other protective equipment. The most likely source of environmental exposure is accidental spills at manufacturing sites and during transport.

4.0 EVALUATION OF AVAILABLE PUBLIC AND COMPANY DATA

4.1 ENVIRONMENTAL FATE DATA

4.1.1 Physicochemical Properties Relevant to Environmental Fate

In order to understand the environmental fate of a substance, one must understand how that substance and its degradation by-products partition among environmental compartments (i.e., air, soil, sediment, suspended sediment, water, and biota). The

physicochemical properties of a substance influence the way in which a substance will degrade. The important environmental degradation pathways are biodegradation, hydrolysis, and photodegradation. Biodegradation is a measure of the potential of compounds to be degraded by microorganisms. Hydrolysis is a reaction in which a water molecule or hydroxide ion substitutes for another atom or group of atoms present in an organic molecule. Photodegradation is the degradation of a chemical compound as a result of absorption of solar radiation.

The physicochemical properties of the parent substance and its degradation by-products will also influence the way in which these substances will partition among environmental compartments. Substances characterized by a low vapor pressure do not partition into air to any great extent. Similarly, substances that are characterized by low water solubility do not partition extensively into water. Substances that do not partition into air and water to any great extent tend to partition into soil and sediments.

4.1.2 Biodegradability

4.1.2.1 Test Methodologies

Chemical biodegradation involves a series of microbially-mediated reactions that may require many kinds of microorganisms acting together to degrade the parent substance. There are several standard test methods, which measure primary degradation (i.e., loss of parent chemical) or ultimate degradation (i.e., complete utilization of the substance to produce carbon dioxide, water, mineral salts, and microbial biomass). Primary degradation can be determined analytically by measuring dissolved organic carbon (DOC) for water-soluble chemicals, infrared absorbance, or by a chemical-specific detection method. Ultimate degradation (also called mineralization) can be determined by measuring oxygen consumption or carbon dioxide evolution relative to the theoretical levels that can be achieved based on an elemental analysis of the chemical under investigation.

4.1.2.2 Summary of Available Data

Biodegradation data for the petroleum additive alkaryl sulfonate category is summarized in Table 4. Two members of the category and one structural analog' have been adequately tested.

Two substances were evaluated for biodegradability under the conditions of the *Manometric Respirometry Test* (OECD Guideline 301F). In the 28-day test for the petroleum derived calcium salt (CAS # 61789-86-4), the extent of biodegradation was 8.6% based on theoretical oxygen demand (ThOD). For the mixed mono-C15-C30 and di-C 11 -C 13 alkaryl calcium salt, overbased derivative (CAS # 71486-79-8), the extent of biodegradation in the 28-day test was 8.6% based on ThOD.

[1] An analog is an alkaryl sulfonate containing the same metal salt of the particular category member and an alkyl chain length that is within the range of the chain length of that category member.

A C20-C24 alkaryl calcium salt derivative (no CAS #) analog of the C16-C24 alkaryl calcium salt salt (CAS # 70024-69-o) was evaluated for biodegradability under the conditions of the *Closed Bottle Test* (OECD Guideline 301D). In the 28-day test, the extent of biodegradability was 8% based on ThOD.

4.1.2.3 Data Assessment and Test Plan for Biodegradability

In total, five biodegradation tests have been conducted on two of the twelve members of the petroleum additive alkaryl sulfonate category and three structural analogs. The alkyl side chains of these five substances are predominantly branched, and the results indicate that these substances are poorly biodegradable.

The HPV Challenge Program requires that a biodegradation test be performed or bridged to each member of a category. Adequate biodegradation data exist for two of twelve substances in the petroleum additive alkaryl sulfonate category. Additional testing and bridging will be used to fill the remaining data gaps for the other ten substances.

- A biodegradability test will be conducted on Cl 5-C30 alkaryl sodium salt derivative (CAS # 78330-12-8), a substance that has predominantly linear alkyl side chains. The results will be bridged to C 16-C24 alkaryl calcium salt derivative (CAS # 70024-69-O), which also has predominantly linear alkyl side chains and has similar physicochemical properties.
- Biodegradation data for petroleum derived calcium salt (CAS # 61789-86-4) will be bridged to the other three petroleum-derived substances in the category, which have similar chemical structures and physicochemical properties:
  - petroleum derived barium salt (CAS # 61790-48-5),
  - petroleum derived sodium salt (CAS # 68608-26-4), and
  - petroleum derived calcium salt, overbased (CAS # 68783-96-O).
- The biodegradation data for mixed mono-C 15-C30 and di-C 11-C 13 alkaryl calcium salt, overbased derivative (CAS # 71486-79-8), which contains both branched and linear alkyl side chains, will be bridged to the other members of the category that also contain branched and liner alkyl side chains and have similar physicochemical properties:
  - mixed mono-C15-C30 and di-Cl 1-C13 alkaryl derivative (CAS # 71549-79-6),
  - alkaryl magnesium salt derivative (CAS # 71786-47-5),
  - C 14-C24 alkaryl calcium salt derivative (CAS # 115733-09-O),
  - C 14-C24 alkaryl calcium salt, overbased derivative (CAS # 115733-10-3), and
  - C14-C24 alkaryl derivative (CAS # 115829-36-2).

4.1.3 Hydrolysis

4.1.3.1 Test Methodologies

The potential for a substance to hydrolyze in water is assessed as a function of pH (OECD Guideline 111, Hydrolysis *as* a *Function of pH*[2]). When an organic molecule undergoes hydrolysis, a nucleophile (water or hydroxide ion) attacks an electrophile and displaces a leaving group (e.g., halogen, phenoxide). [3] Potentially hydrolyzable groups include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters[4]. The lack of a suitable leaving group renders compounds resistant to hydrolysis.

4.1.3.2 Summary of Available Data

There are no published or unpublished hydrolysis studies for members of the petroleum additive alkaryl sulfonate category. An evaluation of the hydrolytic potential of the functional groups in each substance in this category is presented in Tables 4 and 5. Substances derived from the same alkaryl sulfonic acid are grouped together in Table 5.

The twelve substances in the petroleum additive alkaryl sulfonate category do not contain functional groups that are subject to hydrolytic reactions. Desulfonation of the aromatic sulfonic acids and the corresponding salts into sulfuric acid and the aromatic hydrocarbon requires heating to 100 – 175 degrees C in dilute aqueous acid. These conditions would not be typically encountered in the environment. Thus, while the substances in the category that are salts may dissociate in water, all these substances have little, if any, potential for hydrolysis.

4.1.3.3 Data Assessment and Test Plan for Hydrolysis

Since these substances do not contain functional groups that are susceptible to hydrolytic degradative mechanisms[4], testing these substances for hydrolysis as a function of pH is not needed to adequately evaluate this endpoint. Therefore, no hydrolysis testing is proposed for the HPV Challenge Program

4.1.4 Photodegradation

4.1.4.1 Test Methodologies

A prerequisite of photodegradation is the ability of one or more bonds of a chemical to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer.

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[2] Organization for Economic Cooperation and Development (OECD) (1993) OECD Guidelines for Testing of Chemicals. OECD. Paris, France.

[3] W. Lyman et al. (1990) *Handbook of Chemical Estimation Methods.* Chapter 8.

[4] W.J. Lyman, W.F. Reehl, and D.H. Rosenblatt. (1982) Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co. New York, NY, USA.

The Atmospheric Oxidation Potential (AOP) of a substance can be characterized using the modeling program AOPWIN. This computer simulation is recommended in the Agency's recently released structure activity review (SAR) guidance for HPV chemicals.

4.1.4.2 Summary of Available Data

There are no published or unpublished photodegradation studies for members of the petroleum additive alkaryl sulfonate category.

None of the members of the petroleum additive alkaryl sulfonate category contain bonds that have a high potential to absorb UV light above 290 nm. These substances also have low vapor pressure, which indicates that they should not partition into the air to a significant extent.

4.1.4.3 Data Assessment and Test Plan for Photodegradation

The HPV Challenge Program requires a photodegradation test be performed or bridged to each member of a category. The Atmospheric Oxidation Potential (AOP) of these substances will be characterized using the modeling program AOPWIN. The AOP data for representative structures of the category will be evaluated to estimate (1) rate constants for the atmospheric, gas phase reaction as mediated by photochemically produced hydroxyl radicals and (2) atmospheric half- lives based on hydroxyl radical attack.

4.1.5 Fugacity Modeling

4.1.5.1 Modeling Methodologies

Fugacity-based multimedia fate modeling compares the relative distribution of chemicals among environmental compartments. A widely used model for this approach is the EQC model[5].

There are multiple levels of the EQC model. In the document, "Determining the Adequacy of Existing Data", EPA states that it accepts Level I fugacity modeling to estimate transport/distribution values. The Agenc y states that Level III model data are considered "more realistic and useful for estimating a chemical's fate in the environment on a regional basis". The EQC Level I model utilizes input of basic chemical properties, including molecular weight, vapor pressure, and water solubility to calculate percent distribution within a standardized environment. EQC Level III uses these parameters to evaluate chemical distribution based on discharge rates into air, water, and soil, as well as degradation rates in air, water, soil, and sediment.

[5] Equilibrium Criterion Model- Enviromnental Modeling Centre as developed by D. Mackay.

4.1.5.2 Summary of Available Data

There are no published or unpublished fugacity-based multimedia fate modeling data for members of the petroleum additive alkaryl sulfonate category. All of the members of this category have low vapor pressure and low water solubility indicating that they will not partition into the air or water to any great extent.

4.1.5.3 Test Plan for Fugacity

The HPV Challenge Program requires that fugacity modeling be performed or bridged to each member of a category. The relative distribution of substances within this category among environmental compartments will be evaluated using the Level I model. Data developed using a Level I model can then be used for simple comparative purposes across several substances. EQC Level III will not be used for this evaluation because appropriate emission levels are as yet unknown. Because of the physical nature of the substances in this category, a Level I dataset will be as equally robust as a Level III dataset and can then be used to assess the partitioning behavior of petroleum additive alkaryl sulfonates in the environment.

Input data to run the EQC Level I model will require an additional computer model to estimate physical/chemical properties from a structure. The model used for this purpose will be EPIWIN, version 3.02[6], which was developed by the Syracuse Research Corporation. EPIWIN includes algorithms for estimating all physical and chemical properties needed for the EQC model.

Fifteen basic chemical structures will be used for this evaluation and will represent the twelve substances in this category. Representative structures will include Cl6 linear, C23 linear, C23 branched, di-C13 branched, C24 linear and C30 linear homologs. In addition, for selected substances, the high and low molecular weight range will be evaluated.

4.2 ECOTOXICOLOGY DATA

4.2.1 Aquatic Ecotoxicity Testing

4.2.1.1 Test Methodologies

Acute aquatic ecotoxicity tests are usually conducted with three species that represent three trophic levels in the aquatic environment: fish, invertebrates, and algae. The fish acute toxicity test (OECD Guideline 203, *Fish*, Acute Toxicity Test) establishes the lethality of a substance to a fish during a 96-hour exposure period. The acute invertebrate test (OECD Guideline 202, *Daphnia sp., Acute immobilization Test and Reproduction Test)* establishes the lethality of a substance to an invertebrate, typically a daphnid *(Daphnia magna),* during a 48- hour exposure period. The alga growth inhibition test (OECD Guideline *20* 1, *Alga, Growth Inhibition Test)* establishes the potential of a

[6] Environmental Science Center- Syracuse Research Corporation- EPI for windows.

substance to inhibit alga growth, typically using the freshwater unicellular green algae, *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum),* during a 96-hour exposure period.

Three test methodologies are commonly used to conduct aquatic toxicity tests; i.e., flow-through, static, and static renewal tests.

Inflow-through tests, organisms are continually exposed to fresh chemical concentrations in each treatment level in the incoming water and there is greater assurance than with other test methods that the exposure levels and water quality remains constant throughout the test. Although flow-through testing is the preferred method, it is only applicable for chemicals that have adequate water solubility for testing.

In *static tests,* organisms are exposed in still water that is not renewed. The chemical is added to the dilution water to produce the desired test concentrations. Test organisms are then placed in the test chambers, and there is no change of water at any time during the test. There is less assurance that the test concentrations test organisms are exposed to will remain constant because test material can be adsorbed onto test chambers, degraded, volatilized, or otherwise changed during the test. Nevertheless, due to limitations of other test systems for non-volatile materials, the static test has been widely used, especially for testing organisms such as algae and *Daphnia.*

The *static-renewal test* is similar to a static test because it is conducted in still water, but the test solutions and control water are renewed periodically, usually every 24 hours. Daily test solution renewal provides a greater likelihood that the exposure concentrations will remain stable throughout the test. This is the preferred method for conducting aquatic toxicity tests for compounds such as the petroleum additive alkaryl sulfonates on fish. Daily renewals cannot be done in the algae test, and usually not in *Daphnia* tests, because the process of separation and replenishment would cause a discontinuity in the alga growth rate and it can stress, coat, or entrap *Daphnia* in any surface film during renewals. OECD considers the use of static test for fish, *Daphnia,* algae and the use of static renewal test for fish to be appropriate for testing poorly soluble chemicals like the petroleum additive alkaryl sulfonates provided that test solution preparation uses water accommodated fraction or water soluble fraction methods.'

4.2.1.2 Test Solution Preparation

Petroleum additive alkaryl sulfonates are poorly water-soluble substances, and it is not possible to prepare exposure solutions for aquatic toxicity testing by direct addition of

[7] Organization for Economic Cooperation and Development (OECD) (2000). Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Environmental Health and Safety Publications, Series on Testing and Assessment No.23, Paris, France.

measured quantities of test material to water. Two methods' are used to prepare solutions of poorly water-soluble materials for aquatic toxicity testing:

- *Water accommodatedfraction* (WAF) – This is a method in which the test solution contains only that fraction of the test material (organic phase) which is retained in the aqueous phase after a period of stirring long enough to reach equilibrium, followed by a sufficient time (l-4 hours) for phase separation. The WAF (aqueous phase) will contain soluble components of the test material at levels that will be dependent on the test material loading (the amount of material added to the aqueous medium). The resulting WAF is used in the aquatic toxicity test. Ideally, a WAF consists of a water-soluble extract of test material, but it can also include a stable micro-emulsion or contain small amounts of suspended matter.

- *Water soluble fraction* (WSF) This is a method in which a WAF is either filtered, centrifuged, or allowed to settle for a greater length of time (24 hours) than with the WAF method to remove suspended matter from the aqueous phase before being used in the aquatic toxicity test.

4.2.1.3 Reporting Toxicity Results

In both WAF and WSF tests, test material concentrations are expressed as loading rates (i.e., defined as the weight of test material added per unit volume of test medium during WAF or WSF preparation) [9]. For fish tests, endpoints can be expressed as median lethal loading rate (LL_{50}) when lethal effects occur to 50% of the test population or in cases where no lethal effects are observed at all loadings tested, LL_0. In both cases, results can be expressed in mg/L and in studies where no lethality is observed, the result is expressed as LL_0 = the highest loading rate tested. For invertebrate and alga tests, endpoints are expressed as median effective loading rate (EL_{50}) or EL_0 in mg/L as discussed above.

Loading rates allow poorly water-soluble complex substances such as the petroleum additive alkaryl sulfonates to be compared to more readily soluble substances and /or pure chemicals on an equal basis. To allow comparison, the toxicity value is expressed as the amount of test material added per unit volume of water when preparing the WAF or WSF.

If test material exposure levels are analytically measured in the test, the endpoints can also be expressed as median lethal concentration (LC_{50}) or median effective concentration (EC_{50}) in mg/L. EC/LC_{50}s are often not reported because it is very difficult to accurately measure test material exposure levels that can be below 1.0 mg/L.

[8] American Society for Testing and Materials (1998) D6081-98, Standard Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation.
[9] Organization for Economic Cooperation and Development (OECD) (1999) Draft Guidance document on Aquatic Toxicity Testing of' Difficult Substances. OECD, France.

NOTE: In this test plan, these results are reported as loading rates (EL/LL), to reflect the current reporting practices for the WAF method used in the tests. In the robust summaries, these data are presented as concentrations (EC/LC) as originally reported even though the test methods employed WAF preparation of test solutions without measurement of test material concentration.

4.2.2 Aquatic Toxicity of the Petroleum Additive Alkaryl Sulfonate Category

In general, the toxicity of a substance to an organism is limited by mechanisms of uptake and movement to target organs. Characteristics such as smaller molecular size and a lesser degree of ionization increase the ability of a substance to passively cross biological membranes. However, the soluble fraction of a compound in water represents the chemical fraction responsible for toxicity to aquatic organisms. Therefore, aquatic toxicity can be limited by the water solubility of a substance.

Data and preliminary modeling information indicates that all members of the petroleum additive alkaryl sulfonate category have low water solubility. The low water solubility suggests that the acute aquatic toxicity of these substances should be low due to limited bioavailability to aquatic organisms. However, the length of the alkyl side chains on these substances will influence their relative water solubility, and, hence, their relative toxicity. Modeling data indicate that the petroleum derived sodium salt (CAS # 68608-26-4) is predicted to be the most water-soluble member of the category and the member most likely to demonstrate potential aquatic toxicity.

4.2.2.1 Summary of Available Data

Acute aquatic ecotoxicity data for the petroleum additive alkaryl sulfonate category is summarized in Table 6. Four members of the category have been tested for acute aquatic toxicity in at least one species. A low order of toxicity was observed across the range of substances from those that contain the shortest alkyl side chain (C14) to the substances that contain the longest ally1 side chains.

4.2.2.1.1 Fish Acute Toxicity

Three of the twelve substances in the category and one structural analog were evaluated for acute toxicity to fish in five studies. Maximum test material loading rates were either 1,000 or 10,000 mg/L. No mortality was observed in any of the studies. Overall, the LL_{50} for these substances was greater than 1000 mg/L indicating a relatively low order of toxicity to fish.

4.2.2.1.2 Invertebrate Acute Toxicity

Three of the twelve substances in the category were evaluated for acute toxicity to daphnids. The maximum test material loading rate was 1,000 mg/L. In general, minimal effects were observed in the studies. Overall, the EL_{50} for these substances was greater than 1000 mg/L indicating a relatively low order of toxicity to daphnids.

4.2.2.1.3 Alga Toxicity

Three of the twelve substances in the category were evaluated for algal growth inhibition. Maximum test material loading rates were either 1,000 or 1,500 mg/L. The results indicate no observed toxicity to algae at or below 1,000 mg/L. In one test, there was an algistatic effect at 1,500 mg/L, which was reversed when the algae were placed in fresh media. Overall, the EL_{50} for these substances was greater than 1000 mg/L indicating a relatively low order of toxicity to algae.

4.2.2.2 Data Assessment and Test Plan for Acute Aquatic Ecotoxicity

In total, eleven adequate acute aquatic ecotoxicity studies have been conducted for the petroleum additive alkaryl sulfonate category. These studies involved three trophic levels of aquatic organisms and evaluated the acute aquatic ecotoxicity of five of the twelve members of the category. The substances tested ranged from ones with the shortest (C14) alkyl side chain to others with the longest alkyl side chains. The data consistently demonstrate a low order of acute aquatic ecotoxicity regardless of the length of the alkyl side chain. The similarity in the low order of toxicity for these substances is consistent with their similar chemical structure and physicochemical properties and supports the scientific justification of these twelve substances as a category within the HPV Challenge Program.

The HPV Challenge Program requires that an acute aquatic ecotoxicity test in fish, invertebrates, and algae be performed or bridged to each member of a category. Adequate data for all three species exist for two of the twelve s&stances. Additional testing and bridging will be used to fill the data gaps for the remaining ten substances.

- Petroleum derived sodium salt (CAS # 68608-26-4), which contains the shortest alkyl side chain (C14) in the category, will be evaluated for acute aquatic toxicity to fish, daphnids, and algae by conducting a limit test at 1000 mg/L. The results of these studies will be bridged to the other sodium salt in the category, which has similar physicochemical properties:
  - C 15-C30 alkaryl sodium salt derivative (CAS # 78330- 12-8).
- The potential acute aquatic toxicity of petroleum derived calcium salt (CAS # 61789-86-4) to fish, daphnids, and algae will be determined by conducting a limit test at 1000 mg/L. The results of these tests will be bridged to the two remaining petroleum-derived substances in the category, which have similar physicochemical properties:
  - petroleum derived barium salt (CAS # 61790-48-5) and
  - petroleum derived calcium salt, overbased (CAS # 68783-96-O).

An additional limit test at 1000 mg/L will be performed on petroleum derived barium salt (CAS # 61790-48-5) as well.

- C 14-C24 alkaryl calcium salt derivative (CAS # 115733-09-o) will be evaluated for acute aquatic toxicity to fish by conducting a limit test at 1000 mg/L. The results of this test and the existing data for acute aquatic toxicity of this substance to daphnids and algae will be bridged to the other members of the category with similar chemical structures and physicochemical properties:

- C14-C24 alkaryl calcium salt, overbased derivative (CAS # 115733-10-3),
- C14-C24 alkaryl derivative (CAS # 115829-36-2), and
- C 16-C24 alkaryl calcium salt derivative (CAS # 70024-69-o).

The existing fish data for the C20-C24 alkaryl calcium salt, overbased derivative (CAS # 70024-7 1-4) analog of Cl 6-C24 alkaryl calcium salt derivative (CAS # 70024-69-o) will be used to define the influence of longer alkyl chain lengths on aquatic toxicity for these substances.

- The existing data for mixed mono-C 15-C30 and di-C 11 -C 13 alkaryl calcium salt, overbased derivative (CAS # 71486-79-8) will be bridged to mixed mono-C15-C30 and di-C 1 I -C 13 alkaryl derivative (CAS # 7 1549-79-6).

4.3 MAMMALIAN TOXICOLOGY DATA

4.3.1 Physicochemical Properties Relevant to Mammalian Toxicity

Lipophilicity generally enhances the ability of chemicals to cross biological membranes. (either by passive diffusion or active transport via carrier proteins) to reach target tissues or receptors in an organism. Although alkaryl sulfonates are relatively large lipophilic compounds, and molecular size may be a critical limiting determinant for absorption, there is evidence in the published literature" that these substances are absorbed. At the same time, the hydrophobic properties of petroleum additive alkaryl sulfonates suggest that, once they are absorbed, they would undergo limited distribution in the aqueous systemic circulation and reach potential target organs in limited concentrations. Biotransformation by mixed function oxidases often increases the water solubility of a substance, and data in the published literature [11] suggests that these substance undergo oxidation to metabolites with shorter alkyl side chains, which would enhance hydrophilicity. Finally, a chemical must have an active functional group that can interact chemically or physically with the target cell or receptor upon reaching it. For alkaryl sulfonates, the sulfonic acid moiety on an aromatic ring represents the only functional group that may have biological activity.

In addition to the general considerations discussed above, the low volatility of the members of this category indicate that, under normal conditions of use or transportation, exposure by the inhalation route is unlikely. In particular, the high viscosity of these substances suggests that it will be difficult to generate high concentration of respirable particles in the air.

Given the general lipophilic characteristic of these substances, the members of the category with the shortest alkyl side chains (C14) are the most likely to penetrate

[10] W.R. Michael, Metabolism of Linear Alkylate Sulfonate and Alkyl Benzene Sulfonate in Albino Rats. Toxicol. App. Pharmacol. 12.473-485 (I 968).

[11] Reference E = W.R. Michael, Metabolism of Linear Alkylate Sulfonate and Alkyl Benzene Sulfonate in Albino Rats. Toxicol. App. Pharmacol. 12, 473-485 (1968).

biological membranes. Despite the low water solubility of these substances, members of the category with the shortest alkyl side chain would also be the most hydrophilic and the most likely to be distributed in the systemic circulation and possibly reach a potential target organ.

4.3.2 Acute Mammalian Toxicity of the Petroleum Additive Alkaryl Sulfonate Category

4.3.2.1 Acute Toxicity Test Methodology

Acute toxicity studies investigate the effect(s) of a single exposure to a relatively high dose of a substance. Potential routes of exposure for acute toxicity assays include oral, dermal, and inhalation. Oral toxicity assays are conducted by administering test material to fasted animals (typically rats or mice) in a single gavage dose. Acute dermal toxicity tests are conducted by administering test material to the shaved skin on the back of the test animal (typically rats or rabbits) and allowing the test material to stay in contact with the skin application site for a specific duration (usually 24 hours). Acute inhalation toxicity assays are conducted by exposing test animals (typically rats) in a controlled atmosphere to a fixed air concentration of the test substance for a specific duration (typically 4 hours). The test material is either generated as a vapor or intentionally aerosolized into respirable particles, then metered into the exposure air at the desired concentration. Preferably, inhalation toxicity studies are conducted using either nose-only or head-only exposure to minimize potential confounding effects resulting from whole-body exposure. Whole body exposure may lead to over-prediction of inhalation toxicity hazard by increasing the body-burden of the test material through skin absorption or ingestion of test material as a consequence of grooming both during and after the inhalation exposure period.

Historically, lethality is a primary end-point of concern in acute toxicity studies, and the traditional index of oral and dermal potency is the median lethal dose that causes mortality in 50 percent of the test animals (LD_{50}). In acute inhalation studies, the traditional measurement of potency is the median lethal concentration of the test material in air that causes mortality in 50 percent of the test animals (LC_{50}). In addition to lethality, acute toxicity studies also provide insights regarding potential systemic toxicity through careful observation and recording of clinical signs and symptoms of toxicity as well as through detailed examination of tissues and organ systems.

Typically, acute oral and dermal toxicity studies are conducted using a limit dose of 5000 and 2000 mg/kg body weight, respectively, and acute inhalation toxicity studies are conducted using a limit dose of 5 mg/L for 4 hours (according to OECD and EPA testing guidelines). Prior to 1990, some acute dermal toxicity studies may have used a limit dose of 5000 mg/kg. Recently, harmonized EPA testing guidelines (August 1998) have set the limit dose for both oral and dermal acute toxicity studies at 2000 mg/kg body weight, while the recommended limit concentration for acute inhalation studies has been set at 2 mg/L for 4 hours. The limit dose test method minimizes the number of animals tested by exposing a single group of animals to a large dose (the limit dose) of the test substance. A

test substance that shows little or no effects at the limit dose is considered essentially nontoxic, and no further testing is needed. If compound-related mortality is observed at the limit dose, then further testing may be necessary.

4.3.2.2 Summary of Available Data

Acute toxicity data for the petroleum additive alkaryl sulfonate category is summarized in Table 7. Eight members of the category and one structural analog that have been tested for acute oral toxicity have a low order of toxicity. In addition, the three members of the category plus one structural analog tested for acute dermal toxicity and the one member of the category tested for acute inhalation toxicity also have a low order of toxicity. Thus, a low order of toxicity was observed across the range of substances from those that contain the shortest (C14) alkyl side chain to the substances that contain the longest alkyl side chains.

4.3.2.2.1 Acute Oral Toxicity

Eight of the twelve substances in the petroleum additive alkaryl sulfonate category and a C20-C24 alkaryl calcium salt derivative (no CAS #) analog of the C16-C24 alkaryl calcium salt (CAS # 70024-69-o) have been adequately tested for acute oral toxicity (OECD Guideline 401, *Acute Oral Toxicity*). In all but one of these studies, there were no deaths that could be attributed to treatment with the test material when administered at the limit dose of 2000 or 5000 mg/kg. In some studies, the primary clinical observations were diarrhea and reduced food consumption (without a change in body weight). These effects are consistent with the gastrointestinal actions of a detergent in an oil-based vehicle. In other studies, decreased body weight gain or ruffled fur was observed. In one study where deaths occurred, animals were administered dose levels well above the 2000 mg/kglimit dose. Overall, the acute oral LD_{50} for these substances was greater than the 2000 mg/kg limit dose indicating a relatively low order of toxicity.

4.3.2.2.2 Acute Dermal Toxicity

Three of the twelve substances in the petroleum additive alkaryl sulfonate category and a C20-C24 alkaryl calcium salt derivative (no CAS #) analog of the C16-C24 alkaryl calcium salt salt (CAS # 70024-69-o) have been adequately tested for acute dermal (OECD Guideline *402, Acute Dermal Toxicity).* No mortality was observed for any substance when administered at the limit dose of 2000 or 5000 mg/kg. The principal clinical observation was erythema and/or edema at the sight of dermal application. In some cases, the cutaneous findings included dry, flaky skin, desquamation and hyperkeratosis. Overall, the acute dermal LD_{50} for these substances was greater than the 2000 mg/kg limit dose indicating a relatively low order of toxicity.

4.3.2.2.3 Acute Inhalation Toxicity

One member of the petroleum additive alkaryl sulfonate category was tested for acute inhalation toxicity (OECD Guideline *403, Acute Inhalation Toxicity).* Rats were exposed

whole-body to an aerosol of the substance at a nominal atmospheric concentration of 1.9 mg/L for four hours. This was the maximum attainable concentration due to the low volatility and high viscosity of the test material. No mortality was noted, and all animals fully recovered following depuration. Clinical signs of toxicity during exposure included reduced activity, matted coat, and closed eyes. Clinical signs of toxicity observed post exposure included lacrimation, nasal discharge, salivation, rales, matted coat, hunched appearance, soft stools and closed eyes. No treatment-related macroscopic findings were noted. The lack of mortality at a concentration just below the limit dose of 2.0 mg/L indicates a relatively low order of toxicity for this substance.

4.3.2.3 Data Assessment and Test Plan for Acute Mammalian Toxicity

In total, fourteen adequate acute toxicity studies have been conducted for the petroleum additive alkaryl sulfonate category. These studies involved two species of laboratory animals (rats or rabbits); three routes of exposure (oral, dermal, and inhalation); and evaluated the toxicity of eight of the twelve members of the category and one structural analog. The substances tested ranged from ones with the shortest (C14) alkyl side chain to others with the longest alkyl side chains. The data consistently demonstrate a low order of acute toxicity regardless of the length of the alkyl side chain. The similarity in the low order of toxicity for these substances is consistent with their similar chemical structure and physicochemical properties and supports the scientific justification of these twelve substances as a category within the HPV Challenge Program.

The HPV Challenge Program requires that either an acute oral (preferable), dermal, or inhalation test be performed or bridged to each member of a category. Adequate acute oral toxicity tests exist for eight of the twelve substances in the petroleum additive alkaryl sulfonate category. Bridging will be used to fill the data gaps for the remaining four substances.

- Acute oral toxicity data for C14-C24 alkaryl calcium salt derivative (CAS # 115733-09-o) will be bridged to the other members of the category with similar chemical structures and physicochemical properties:
  - C14-C24 alkaryl acid derivative (CAS # 115829-36-2),
  - C 14-C24 alkaryl calcium salt, overbased derivative (CAS # 115733-10-3),
  - Cl 6-C24 alkaryl calcium salt derivative (CAS # 70024-69-O),
- Acute oral toxicity data for C 14- C24 alkaryl calcium salt derivative (CAS # 115733-09-0), which contains the shortest alkyl side chains in the category, will also be bridged to the remaining substance in the category, which has the longest alkyl side chains, due to the lack of an upward trend in toxicity over the range of alkyl side chain lengths that have been tested:
  - mixed mono-C15-C30 and di-C1 1-C13 alkaryl calcium salt, overbased derivative (CAS # 71486-79-8).

By bridging these data to the four untested substances, the acute toxicity of the category has been evaluated adequately with respect to all acute toxicity endpoints, and no additional acute toxicity testing is proposed for the HPV Challenge Program

4.3.3 Mutagenicity of the Petroleum Additive Alkaryl Sulfonate Category

4.3.3.1 Mutagenicity Test Methodology

Genetic toxicology is concerned with the effects of substances on genetic material (i.e., DNA and chromosomes). Within genetic material, the gene is the simplest functional unit composed of DNA. Mutations are generally nonlethal, heritable changes to genes which may arise spontaneously or as a consequence of xenobiotic exposure. Genetic mutations are commonly measured in bacterial and mammalian cells. The simplest test systems measure the occurrence of a base-pair substitution mutation in which a single nucleotide is changed followed by a subsequent change in the complementary nucleotide on the other DNA strand. Frame shift mutations occur following the deletion or insertion of one or more nucleotides, which then changes the "reading frame" for the remainder of the gene or multiple genes. Genetic testing for these types of point mutations is generally accomplished by *in vitro* cellular assays for forward or reverse mutations. A forward mutation occurs when there is a detectable change in native DNA whereas a reverse mutation occurs when a mutated cell is returned to its initial phenotype. Both base-pair substitutions and frame shift mutations are routinely measured in bacterial cells by measuring the ability of a cell to acquire the capability to grow in an environment missing an essential amino acid. In these tests, a large number of cells are examined to demonstrate a significant increase in the frequencies of mutations that occur over the frequency of spontaneous mutations.

Chromosomal aberrations are large scale numerical or structural alterations in eukaryotic chromosomes including deletions (visualized as breaks), translocations (exchanges), non-disjunction (aneuploidy), and mitotic recombination. Chromosomal breakage is the classical end point in chromosomal aberration assays. Substances that induce structural changes in chromosomes, especially chromosome breaks, are referred to as "clastogens." To visualize chromosomes and chromosomal aberrations following *in vitro* or in *vivo* treatment with a substance, cells are arrested in metaphase, treated to swell the chromosomes, fixed, transferred to slides and stained. The first metaphase following treatment is the time at which the greatest number of cells with damaged chromosomes may be observed. The most frequently used test systems investigate changes in mammalian cells (such as Chinese hamster ovary or lung cells; human or rat lymphocytes; or human. rat or mouse bone marrow cells) following either in *vitro* or *in vivo* exposure to the test substance. The micronucleus test is a common *in vivo* assay that measures the frequency of micronuclei formation (i.e., chromosomal fragments) in polychromatic erythrocytes.

4.3.3.2 Summary of Mutagenicity Data

A summary of the mutagenicity information for the petroleum additive alkaryl sulfonate category is presented in Table 8. Either bacterial or mammalian gene mutation assays, in *vitro* chromosomal aberration assays, or *in vivo* chromosomal aberration assays have been conducted for two of the twelve members of the category and two structural analogs. Neither mutagenicity nor clastogenicity was exhibited by any of the substances

in the referenced tests with or without metabolic activation. Neither mutagenicity nor clastogenicity was observed across the range of materials from those that contain the shortest (C14) alkyl side chain to the substances that contain the longer alkyl side chains.

4.3.3.2.1 Bacterial Gene Mutation Assay

Two of the twelve substances in this category and two structural analogs, a C20-C24 alkaryl calcium salt derivative (no CAS #) analog of the C16-C24 alkaryl calcium salt (CAS # 70024-69-o) and a C15-C21 alkaryl sodium salt derivative (no CAS #) analog of C 15-C30 alkaryl sodium salt derivative (CAS # 78330-12-8), have been adequately tested *in* a *Bacterial Reverse Mutation Test* (OECD Guidelines 471 and/or 472). All tested substances were negative for mutagenic activity, with and without metabolic activation.

4.3.3.2.2 Mammalian Gene Mutation Assay

One substance in this category has been adequately tested in a mouse lymphoma cell assay (OECD Guideline 476, In *Vitro Mammalian Cell* Gene *Mutation Test).* The results of this study were negative for mutagenic activity with and without metabolic activation of the test substance.

4.3.2.2.3 *In vivo* Chromosomal Aberration Assays

Two of the twelve substances in this category and a C20-C24 alkaryl calcium salt derivative (no CAS #) analog of the C 16-C24 alkaryl calcium salt (CAS # 70024-69-o) have been adequately tested in an *in vivo* chromosomal aberration assay. These studies were conducted using bone marrow cells from mice that were dosed by oral gavage or intraperitoneal injection (OECD Guideline 474, *Mammalian Erythrocyte Micronucleus Test).* All test substances were negative for clastogenicity.

4.3.2.2.4 *In* vitro Chromosomal Aberration Assay

Two substances have been adequately tested in an *in vitro* chromosomal aberration assay using Chinese hamster ovary cells (OECD Guideline 473, *In Vitro Mammalian Chromosome Aberration Test).* The results of these studies, performed with and without metabolic activation of the test material, were negative for clastogenicity.

4.3.3.3 Data Assessment and Test Plan for Mutagenicity

Two of the twelve members of the petroleum additive alkaryl sulfonate category and two structural analogs have been tested for mutagenicity in ten tests for gene mutations and chromosomal aberrations. The assays included point mutations in bacterial or mammalian cells, *in vitro* chromosomal aberrations in mammalian cells, and *in vivo* chromosomal aberrations in mice. The substances tested ranged from one with the shortest (C14) alkyl side chain to others with the longest alkyl side chains. The data consistently demonstrate no evidence of genotoxicity regardless of the length of the alkyl side chain. This suggests

that all members of the category lack genotoxicity due to their similarity in chemical structures and physicochemical properties and supports the scientific justification of these twelve substances as a category within the HPV Challenge Program.

The HPV Challenge Program requires that a gene mutation and a chromosomal aberration test be performed or bridged to each member of a category. Adequate gene mutation and chromosomal aberration tests exist for two of the twelve substances in the petroleum additive alkaryl sulfonate category and one structural analog. Bridging will be used to fill the data gaps for the remaining ten substances.

- Data for petroleum derived calcium salt overbased (CAS # 68783-96-O) will be bridged to the other three petroleum-derived substances in the category:
  - petroleum derived calcium salt (CAS # 61789-86-4),
  - petroleum derived sodium salt (CAS # 68608-26-4), and
  - petroleum derived barium salt (CAS # 6 1790-48-5).
- Data for petroleum derived calcium salt overbased (CAS # 68783-96-O) will also be bridged to the other seven members of the category, which have similar chemical structures and physicochemical properties:
  - C14-C24 alkaryl calcium salt derivative (CAS # 115733-09-O),
  - C14-C24 alkaryl calcium salt, overbased derivative (CAS # 115733-10-3),
  - C14-C24 alkaryl acid derivative (CAS # 115829-36-2),
  - C15-C30 alkaryl sodium salt derivative (CAS # 78330-12-8),
  - mixed mono-C I 5-C30 and di-C I I -Cl3 alkaryl derivative (CAS # 71549-79-6),
  - mixed mono-C I 5-C30 and di-C 11 -C 13 alkaryl calcium salt, overbased derivative (CAS # 71486-79-8), and
  - C16-C24 alkaryl calcium salt derivative (CAS # 70024-69-o).

This is justified since there is no upward trend in genotoxicity between the petroleum derived calcium salt overbased, which contains the shortest alkyl side chain (C14), and the C20-C24 alkaryl calcium salt derivative (no CAS #) analog of C16-C24 alkaryl calcium salt (CAS # 70024-69-0), which defines the genotoxicity of the members of the category with the longer alkyl side chain lengths.

By bridging these data IO the ten untested substances, the category has been evaluated adequately for genotoxicity, and no additional testing is proposed for the HPV Challenge Program.

4.3.4 Repeated-dose Toxicity of the Petroleum Additive Alkaryl Sulfonate Category

4.3.4.1 Repeated-dose 'Toxicity Test Methodology

Repeated-dose toxicity studies evaluate the systemic effects of repeated exposure to a chemical over a significant period of the life span of an animal (rats, rabbits, or mice). Chronic repeated-dose toxicity studies are concerned with potential adverse effects upon exposure over the greater part of an organism's life span (e.g., one to two years in rodents). Subchronic repeated-dose studies are also concerned with effects caused by exposure for an extended period, but not one that constitutes a significant portion of the

expected life span. Subchronic studies are useful in identifying target organ(s), and they can be used in selecting dose levels for longer-term studies. Typically, the exposure regimen in a subchronic study involves daily exposure (at least 5 consecutive days per week) for a period of at least 28 days or up to 90 days (i.e., 4 to 13 weeks). A recovery period of two to four weeks (generally included in most study designs) following completion of the dosing or exposure period provides information on whether or not the effects seen during the exposure period are reversible upon cessation of treatment. The dose levels evaluated in repeated-dose toxicity studies are notably lower than the relatively high limit doses used in acute toxicity studies. The NOAEL (no observed adverse effect level), usually expressed in mg/kg/day, defines the dose of test material that produced no significant toxicological effects. If the test material produce toxicity at the lowest dose tested (i.e., there is no defined NOAEL), the lowest dose that produced an adverse effect is defined as the LOAEL (lowest observed adverse effect level). While these studies are designed to assess systemic toxicity, the study protocol can be modified to incorporate evaluation of potential adverse reproductive and/or developmental effects.

Reproductive and developmental toxicity studies generate information on the effects of a test substance on male and female reproductive performance such as gonadal function, mating behavior, conception, and development of the conceptus, parturition, and post-partum development of the offspring. Various study designs exist, but they all involve exposure to both male and female test animals before mating. The rat is most often selected as the test species. The test substance is administered to males and females continuously at several graduated doses for at least two weeks prior to mating and until the animals are sacrificed. The males are treated for at least two more weeks. Male gonadal histopathology is carefully assessed at the end of the study. The females are treated through parturition and early lactation. The adult females and offspring are typically studied until termination on post-natal day 21, or sometimes earlier. In addition to providing data on fertility and reproduction, this study design provides information on potential developmental toxicity following prenatal and limited post-natal exposure to the test substance. An NOAEL or LOAEL is also used to describe the results of these tests, with the exception that these values are derived from effects specific to reproduction or development.

The "toxicity to reproduction" requirement in the HPV Challenge Program can be met by conducting the *Reproduction/Developmental Toxicity Screening Test* (OECD Guideline 421) or by adding this screening test to a repeated-dose study (OECD Guideline 422, *Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test).* The *One-Generation Reproduction Toxicity Study* (OECD Guideline 41.5) is a more comprehensive protocol for the study of the effect of a test material on reproduction and development that also meets the OECD SIDS and the HPV Challenge Program requirements.

4.3.4.2 Summary of Repeated-Dose Toxicity Data

A summary of the results from the repeated-dose studies for the petroleum additive alkaryl sulfonate category is presented in Table 9. Repeated-dose toxicity tests have been

performed on three members of the petroleum additive alkaryl sulfonate category by three routes of administration and in two species of laboratory animals, rats and rabbits. Repeated oral administration to rats caused decreased serum cholesterol levels at the highest dose tested, 1000 mg/kg/day. Repeated inhalation exposure to rats caused adverse effects on the lungs. Repeated dermal administration to rats and rabbits caused inflammatory skin changes following repeated skin contact, but there were no systemic effects in rats. Systemic effects observed in rabbits following repeated dermal administration include reduction in hematological parameters and evidence of liver injury. The adverse effects on male reproductive organs were observed only in rabbits after repeated dermal application of dose levels that were irritating to the skin.

4.3.4.2.1 Systemic Toxicity Tests

Two of the 12 substances in the alkaryl sulfonate category and a structural analog have been tested for subchronic toxicity in five studies.

Petroleum derived calci um salt, over-based (CAS # 68783-96-O) was evaluated in a 28-day repeated-dose dermal toxicity study in rats (OECD Guideline 410, *Repeated Dose Dermal Toxicity: 21/28 Day*). The substance was applied topically at doses of 100, 300, and 1000 mg/kg/day under occlusive dressing six hours/day for 28 consecutive days. A low incidence of erythema, desquamation and scabbing was sporadically observed in treated animals. The NOAEL for systemic toxicity for this study was 1000 mg/kg/day.

Petroleum derived calcium salt, overbased (CAS # 68783-96-O) was also evaluated in a 28-day inhalation toxicity study in rats (OECD Guideline 412, *Repeated Dose Inhalation Toxicity: 28/14 Day).* Inhalation exposures were six hours/day, five days/week for four weeks at actual whole-body exposure concentrations of 49.5, 156 and 260 mg/m^3. The experimental animals w cre observed to have red nasal discharge, matted coat and decreased activity at the two highest dose levels. Dose-related increases in lung weight were accompanied by microscopic evidence of intralobular macrophage accumulation and bronchiole epithelial hyperplasia/hypertrophy. Based on the latter findings, the NOAEL was 49.5 mg/m^3.

A C20-C24 alkaryl calcium salt derivative (no CAS #) analog of C16-C24 alkaryl calcium salt derivative (CAS # 70024-W-O) was evaluated in a 28-day repeated-dose oral toxicity study in rats (OECD Guideline 407, *Repeated Dose 28-Day Oral Toxicity Study in Rodents).* The substance was administered at 100, 500 and 1000 mg/kg/day for 28 consecutive days. Serum chemistry analysis revealed significant reductions in cholesterol in the high dose male and female groups. Based on the reduction in mean serum cholesterol, the NOAEL was 500 mg/kg/day.

Alkaryl magnesium salt derivative (CAS # 7 1786-47-5) was evaluated in a 28-day repeated-dose dermal toxicity study in rats (OECD Guideline 410). The substance was applied topically at doses of 100, 300 or 1000 mg/kg under occlusive dressing six hours/day for 28 consecutive days. Local cutaneous responses, characterized by

desquamation and hypcrkeratosis were seen in some rats. The NOAEL for systemic toxicity for this study was 1000 mg/kg/day.

Alkaryl magnesium salt derivative (CAS # 7 1786-47-5) was also evaluated in a 28-day repeated-dose dermal toxicity study in rabbits (OECD Guideline 410). The substance was applied topically at a dose volume of 2 ml/kg/day and concentrations of 0, 25, or 100% (w/v) in Primol 205 for six hours/day, five days/week for 20 days. Local irritation responses (i.e., edema, erythema, desquamation, fissuring, and hyperkeratosis) were observed at the site of treatment in both dose groups. Systemic findings included significant reductions in hematological parameters (hemoglobin, hematocrit, erythrocyte count and leukocyte count) in the high dose group. Reduction in total plasma protein (including globulin) and increases in serum alkaline phosphatase, SGPT, and SGOT levels were observed in both treatment groups. Elevations in the serum levels of hepatic enzymes were accompanied by increases in liver weights in both treatment groups, but histopathological lesions (multifocal hepatocellular degeneration) were observed only in the high dose group. Testes and epididymides weights were also reduced in both treatment groups. Microscopic changes observed in the high dose group included aspermatogenesis and multifocal tubular hypoplasia in the testes and epithelial hypoplasia in the epididymides. Due to the observation of adverse effects at both dose levels, an NOAEL was not established in this study.

4.3.4.2.2Reproductive/Developmental Toxicity

No reproductive or developmental toxicity data considered adequate under the HPV Challenge Program are available for the petroleum additive alkaryl sulfonate category.

4.3.4.2Data Assessment and Test Plan for Repeated-dose Toxicity

Five repeated-dose toxicity studies using two different animal species, rats and rabbits, have been conducted with two of the twelve category members and one structural analog of a substance in this category. The substances tested ranged from one with the shortest (C14) alkyl side chain to others with the longer alkyl side chains. In rats, repeated oral administration caused minimal systemic toxicity. Repeated dermal application caused local skin injury at the site of application. and repeated inhalation caused local injury to the lungs. In rabbits, repeated dermal application caused severe skin irritation, liver toxicity, and reproductive organ toxicity that was not observed in rats. The liver toxicity, which occurred only at the high dose level in rabbits, may have been due to the differences in dose selection between the rat and rabbit studies. The high dose in the rat studies was the limit dose of 1000 mg/kg/day, but the high dose in the rabbit study, approximately 2.28 g/kg/day, exceeded the limit dose. The adverse effects on male reproductive organs appear to be a specific response of the rabbit to the effects of severe cutaneous irritation rather than a systemic response to a toxic xenobiotic. Changes in male reproductive organs in the rabbit have been observed when other irritating

substances are applied to the skin at dose levels that cause skin lesions. [12,13] These effects appear to be specific to the rabbit, since similar effects were not observed in rats following repeated dermal application of dose levels that were irritating to the skin. Therefore, the data from the rat studies demonstrate no evidence of repeated-dose toxicity, regardless of the length of the alkyl side chain, at dose levels at or below the limit dose of 1000 mg/kg/day.

The HPV Challenge Program requires that a repeated-dose toxicity study and a reproductive toxicity study be performed or bridged to each member of a category. Adequate data for repeated-dose toxicity exist for two of the twelve substances in the petroleum additive alkaryl sulfonate category. Bridging can be used to fill the data gaps for the remaining ten substances.

- The repeated-dose toxicity data for petroleum derived calcium salt, overbased (CAS # 68783-96-O) can be bridged to the other three petroleum-derived substances in the category:
  - petroleum derived calcium salt (CAS # 61789-86-4),
  - petroleum derived sodium salt (CAS # 68608-26-4), and
  - petroleum derived barium salt (CAS # 61790-48-5).
- The repeated-dose toxicity data for petroleum derived calcium salt, overbased (CAS # 68783-96-O) can also be bridged to the other seven members of the category, which have similar chemical structures and physicochemical properties:
  - C 14-C24 alkaryl calcium salt derivative (CAS # 115733-09-O),
  - C 14- C24 alkaryl calcium salt, overbased derivative (CAS # 115733- 1 0-3),
  - C 14-C24 alkaryl acid derivative (CAS # 115829-36-2),
  - C15-C30 alkaryl sodium salt derivative (CAS # 78330-12-8),
  - mixed mono-C15-C30 and di-Cl l-Cl3 alkaryl derivative (CAS # 71549-79-6),
  - mixed mono-C15-C30 and di-Cll-Cl3 alkaryl derivative (CAS # 71486-79-8), and
  - C16-C24 alkaryl calcium salt derivative (CAS # 70024-69-o).

This is justified since there is no upward trend in repeated-dose toxicity between the petroleum derived calcium salt overbased, which contains the shortest alkyl side chain (C14), and the C20-C24 alkaryl calcium salt derivative (no CAS #) analog of C16-C24 alkaryl calcium salt (CAS # 70024-69-O), which defines the toxicity of the members of the category with the longer alkyl side chain lengths.

However, since a reproductive toxicity study will need to be conducted for the alkaryl sulfonate category (as discussed below), a 28-day repeated-dose oral toxicity will be

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[12] Wong, Z. A., VonBurg, R.. Spangler, W. L., and MacGregor, J. A. (1982) Testicular Damage in the Rabbit Resulting from Simple Chemical Cutaneous Irritation. The Toxicologist 2: 4 1.

[13] McKee, R. H., Kapp, Jr., R. W., and Ward, D. P. (1985) Evaluation of the Systemic Toxicity of Coal Liquefaction-Derived Materials Following Repeated Dermal Exposure in the Rabbit. J. App. Toxicol. 5: 345-351.

conducted with the C14-24 alkaryl calcium salt (CAS # 115733-09-o) in order to select appropriate dose levels for the reproductive toxicity study. The data from this study will also better define the repeated-dose toxicity of the category members with the shortest alkyl side chains by the oral route of exposure,

Although the petroleum additive alkaryl sulfonate category is well tested for other health effects, no reproductive or developmental toxicity studies considered to be adequate under the HPV Challenge Program are available for the members of the petroleum addtives alkaryl sulfonate category. Thus, the reproductive toxicity potential of the petroleum additive alkaryl sulfonates will be evaluated with a one-generation test (OECD Guideline 415) using C 14-C24 alkaryl calcium salt derivative (CAS # 115733-09-o) and the results will be bridged to the other members of the category. This material has been selected because it contains the highest proportion of fractions with the shortest alkyl chain length in the category.

FIGURE 1. PERCENTAGE OF EACH CARBON CHAIN NUMBER IN ALKYL SIDE CHAIN

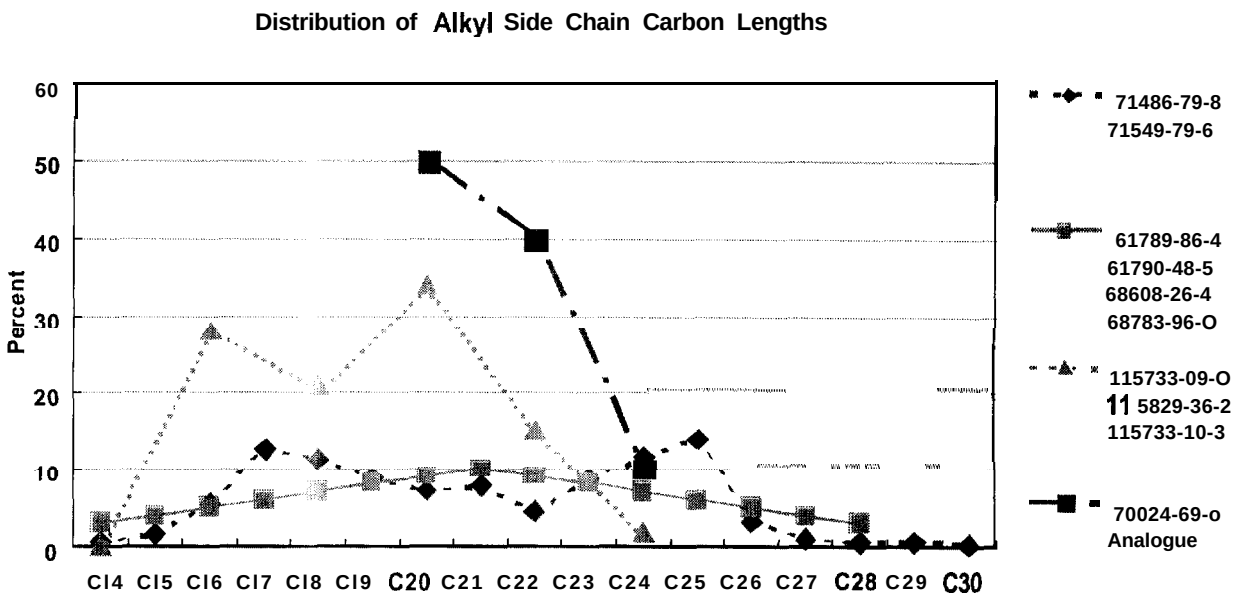

FIGURE 2. GENERAL STRUCTURE OF A PETROLEUM ADDITIVE ALKARYL SULFONATE REVERSE MICELLE

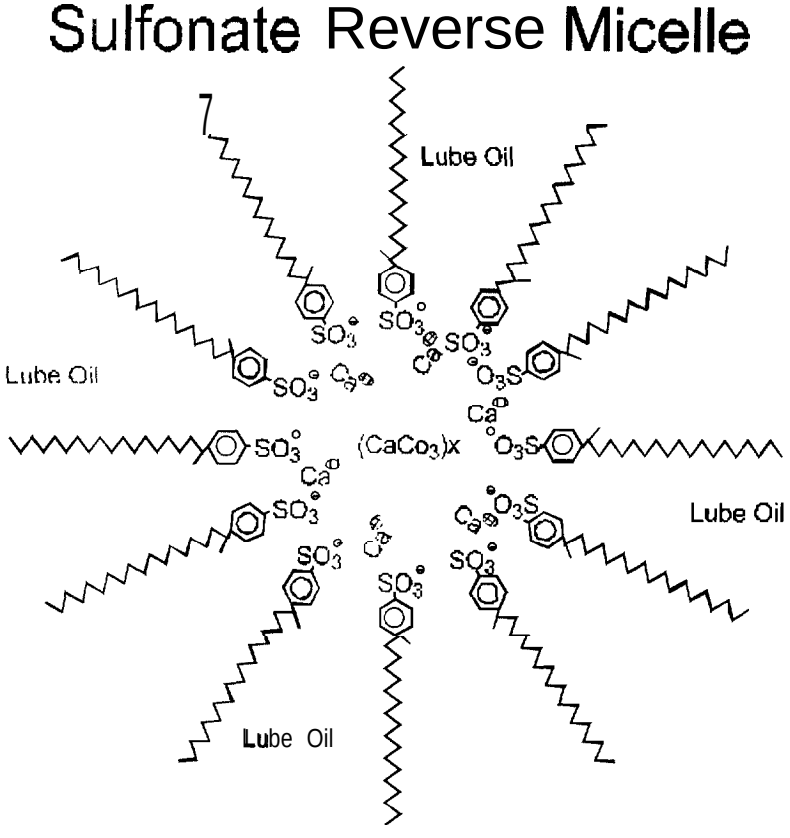

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TABLE 1. MEMBERS OF THE PETROLEUM ADDITIVE ALKARYL SULFIDE CATEGORY

CAS Number	Chemical Name	Simplified Chemical Name
61789-86-4	Sulfonic acids, petroleum, calcium salts	Petroleum derived calcium salt
61790-48-5	Sulfonic acids, petroleum, barium salts	Petroleum derived barium salt
68608-26-4	Sulfonic acids, petroleum, sodium salts	Petroleum derived sodium salt
68783-96-O	Sulfonic acids, petroleum, calcium salts, overbased	Petroleum derived calcium salt, overbased
70024-69-o	Benzenesulfonic acid, mono-C 16-C24 alkyl derivatives, calcium salts	C16-C24 alkaryl calcium salt derivative
71486-79-8	Benzcnesulfonic acid, mono-C15-C30 branched alkyl and di-C11-Cl3 branched and linear alkyl derivatives, calcium salts, overbased	Mixed mono-C 15-C30 and di-C 11-C 13 alkaryl calcium salt, overbased derivative
7 1549-79-6	Benzcnesulfonic acid, mono-C15-C30 branched alkyl and di-C 11 -C 13 branched and linear alkyl derivatives	Mixed mono-C 15-C30 and di-C 11 -C 13 alkaryl derivative
71786-47-5	Benzenesulfonic acid, mono and dialkyl derivn t i ves, magnesium salts	Alkaryl magnesium salt derivative
78330-12-8	Benzcnesulfonic acid, C15-C30 alkyl derivatives, sodium salts	Cl 5-C30 alkaryl sodium salt derivative
115733-09-O	Benzcnesulfonic acid, C14-C24 branched and linear alkyl derivatives, calcium salts	C14-C24 alkaryl calcium salt derivative
115733-10-3	Benzcnesulfonic acid, C 14-C24 branched and linear alkyl derivatives, calcium salts, overbased	C14-C24 alkaryl calcium salt, overbased derivative
115829-36-2	Benzcnesulfonic acid, C14-C24 branched and linear alkyl derivatives	C 14-C24 alkaryl derivative

TABLE 2. CHEMICAL STRUCTURES OF PETROLEUM ADDITIVE ALKARYL SULFONATES

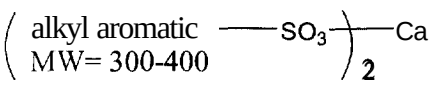
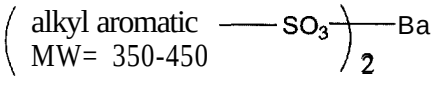
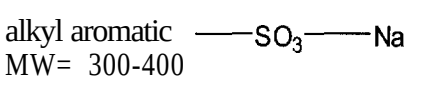
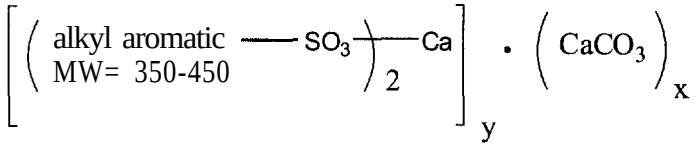
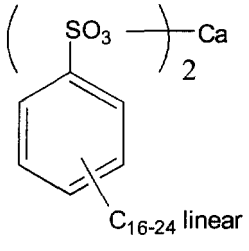
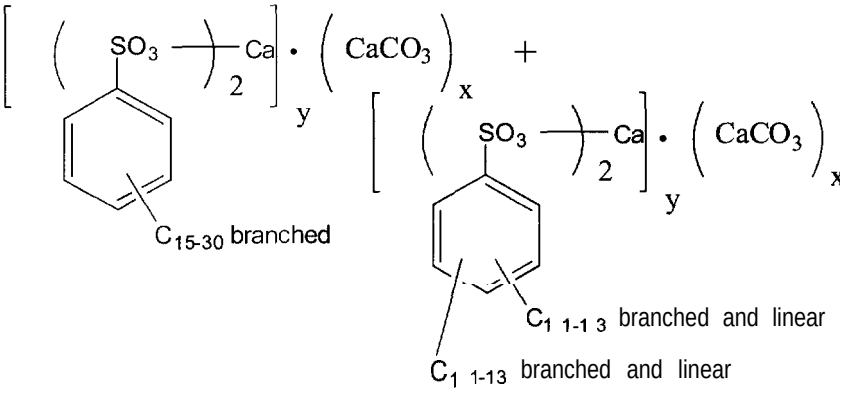
CAS Number	Chemical Structure
61789-86-4	
6 1790-48-5	
68608-26-4	
68783-96-O	
7002469-O	
71486-79-8	

TABLE 2. CHEMICAL STRUCTURE S OF PETROLEUM ADDITIVE ALKYL SULFONATE (CONT.)

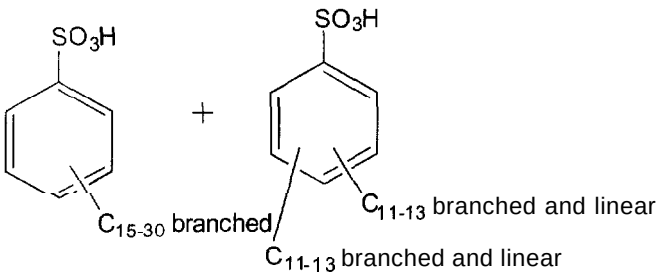
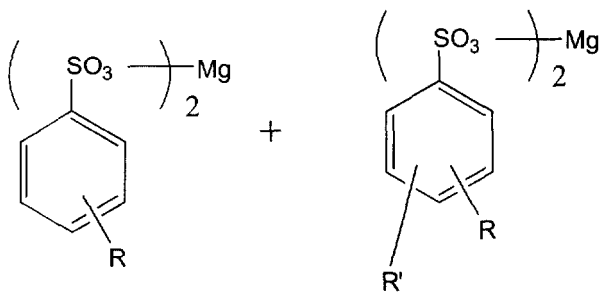
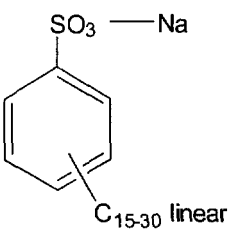
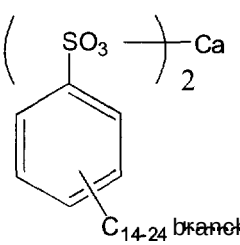
CAS Number	Chemical Structure
71549-79-6	
71786-47-5	 + the mixed mono and dialklybenzenesulfonic acid salts
78330-12-8	
115733-09-0	

TABLE 2. CHEMICAL STRUCTURE S OF PETROLEUM ADDITIVE ALKYL SULFONATE (CONT.)

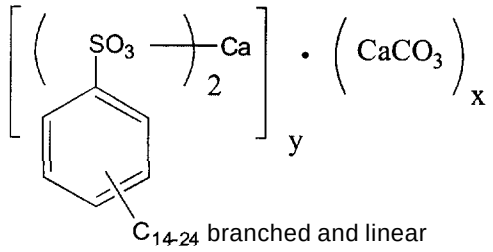
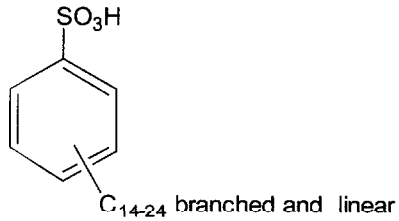
CAS Number	Chemical Structure
115733-10-3	
115829-36-2	

TABLE 3. PHYSICOCHEMICAL PROPERTIES OF PETROLEUM ADDITIVE ALKARYL SULFONATES

CAS Number	Equivalent Weight[1]	Carbon Number Range	Specific Gravity[2] g/ml	Viscosity[3] cSt @ 100 °C	Melting Point[4] °C	Boiling Point[5] °C	Vapor Pressure[6] Pa	Water Solubility mg/L	Log Kow
115829-36-2	354-494	C14-C24	No data	No data	208.45	506.34	$<1X10^{-10}$	No data[7]	No data[7]
115733-09-0	393-533	C14-C24	No data	No data	349.84	935.88	$<1X10^{-10}$	No data[7]	No data[7]
115733-10-3	393-533	C14-C24	No data	No data	349.84	935.88	$<1X10^{-10}$	No data[7]	No data[7]
68608-26-4	376-600	C14-C30	No data	No data	309.31	707.03	$<1X10^{-10}$	No data[8]	No data[7]
61789-86-4	393-617	C14-C30	0.977	175	349.84	935.88	$<1X10^{-10}$	No data[7]	No data[7]
68783-96-0	393-617	C14-C30	1.165	190	349.84	935.88	$<1X10^{-10}$	No data[7]	No data[7]
61790-48-5	490-714	C14-C30	No data	No data	349.84	935.88	$<1X10^{-10}$	No data[7]	No data[7]
78330-12-8	390-600	C15-C30	No data	No data	347.25	788.26	$<1X10^{-10}$	No data[8]	No data[7]
71549-79-6	368-578	C15-C30	No data	No data	208.45	506.34	$<1X10^{-10}$	0.075	>6.7
71486-79-8	407-617	C15-C30	No data	No data	341.76	776.50	$<1X10^{-10}$	No data[7]	No data[7]
70024-69-0	421-533	C16-C24	No data	No data	349.84	935.88	$<1X10^{-10}$	<0.100	>6.0
71786-47-5	461-517	C20-C24	1.142	225	349.84	935.88	$<1X10^{-10}$	No data[7]	No data[7]

[1] **Equivalent weight = molecular weight of one alkylbenzene sulfonic acid plus molecular weight of metal.**

[2] ASTM D1298-99, Standard Test Method for Density, Relative Density (Specific Gravity), or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method

[3] ASTM D 445-97, Standard Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (the Calculation of Dynamic Viscosity)

[4] Modeling data; melting point cannot be measured due to viscosity of liquid.

[5] Modeling data; boiling point cannot be determined because substance decomposes before it boils.

[6] "De-oiled" petroleum additive alkaryl sulfonates are solid. As manufactured, vapor pressure is estimated from the vapor pressure of the petroleum base stock in which the substance is manufactured.

[7] No data needed; bridging from other members of the category.

[8] Testing for water solubility will be conducted.

TABLE 4. EVALUATION OF ENVIRONMENTAL FATE INFORMATION FOR PETROLEUM ADDITIVE ALKARYL SULFONATES

CAS Number	BIODEGRADABILITY Available Data & Proposed Testing	HYDROLYSIS Available Data & Proposed Testing	PHOTODEGRADATION Available Data & Proposed Testing
115829-36-2	No testing needed Bridging	No testing needed'	AOPWIN Model Estimation
115733-09-O	No testing needed Bridging	No testing needed'	AOPWIN Model Estimation
115733-10-3	No testing needed Bridging	No testing needed'	AOPWIN Model Estimation
68608-26-4	No testing needed Bridging	No testing needed'	AOPWIN Model Estimation
61789-86-4	8.6% biodegraded after 28 days	No testing needed'	AOPWIN Model Estimation
68783-96-O	No testing needed Bridging	No testing needed'	AOPWIN Model Estimation
61790-48-5	No testing needed Bridging	No testing needed'	AOPWIN Model Estimation
78330-12-8	Test	No testing needed'	AOPWIN Model Estimation
7 1549-79-6	No testing needed Bridging	No testing needed'	AOPWIN Model Estimation
7 1486-79-8	8.6% biodegraded after 28 days	No testing needed'	AOPWIN Model Estimation
70024-69-o	No testing needed Bridging	No testing needed'	AOPWIN Model Estimation
C20-C24 alkaryl calcium salt derivative (no CAS #) analog of 70024-69-O	8.0% biodegraded after 28 days	No testing needed'	No estimation needed for analogs
71786-47-5	No testing needed Bridging	No testing needed'	AOPWIN Model Estimation

'See technical discussion of information presented in Table 5.

TABLE 5. FUNCTIONAL GROUP, CHEMICAL CLASSES, AND HYDROLYTIC POTENTIAL OF PETROLEUM ADDITIVE ALKARYL SULFONATES

CAS Number	Functional Group and Chemical Class	Potential for Hydrolysis
115829-36-2	Aromatic benzene ring	Low
115733-09-O	Branched hydrocarbon chain	LOW
115733-10-3	Linear hydrocarbon chain	Low
	Sulfonic acid	Low
68608-26-4	Aromatic benzene ring	Low
61789-86-4	Branched hydrocarbon chain	Low
68783-96-O	Linear hydrocarbon chain	Low
6 1790-48-5	S ul fonic acid	Low
78330-12-x	Aromatic benzene ring	LOW
	Linear hydrocarbon chain	LOW
	Sulfonic acid	Low
7 1549-79-6	Aromatic benzene ring	LOW
71486-79-8	Branched hydrocarbon chain	Low
	Linear hydrocarbon chain	Low
	Sulfonic acid	LOW
70024-69-o	Aromatic benzene ring	LOW
	Linear hydrocarbon chain	LOW
	Sulfonic acid	Low
71786-47-5	Aromatic benzene ring	Low
	Branched hydrocarbon chain	LOW
	Linear hydrocarbon chain	Low
	Sulfonic acid	Low

TABLE 6. EVALUATION OF AQUATIC TOXICOLOGY OF PETROLEUM ADDITIVE ALKARYL SULFONATES

CAS Number	ACUTE TOXICITY TO FISH 96-hr LL_{50} (mg/L)[1]	ACUTE TOXICITY TO INVERTEBRATES 48-hr EL_{50} (mg/L)[1]	TOXICITY TO ALGAE 96-hr EL_{50} (mg/L)[1]
	Available Data & Proposed Testing	Available Data & Proposed Testing	Available Data & Proposed Testing
115829-36-2	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
115733-09-0	Limit Test	>1,000 (WAF[3], D)	>1,000 (WAF[3], P, R) >1,000 (WAF[3], P, B)
115733-10-3	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
68608-26-4	Limit Test	Limit Test	Limit Test
61789-86-4	Limit Test on T >10,000 (WAF[2], S)	Limit Test	Limit Test
68783-96-0	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
61790-48-5	Limit Test	No testing needed Bridging	No testing needed Bridging
78330-12-8	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging

[1]Toxicity endpoints are expressed as median lethal loading rates (LL_{50}) for fish and median effective loading rates (EL_{50}) for *Daphnia* and algae. The EL/LL_{50} i defined as the loading rate that adversely effects 50% of the test organisms exposed to it during a specific time. The greater the EL/LL_{50} the lower the toxicity.

[2]WAF = Water accommodated fraction static renewal test.

[3]WAF = Water accommodated fraction static non-renewal test.

[4]EL/LL_0 = no mortality or effects observed at the highest loading rate tested.

F = fathead minnow, *Pimephales promelas.*

D = freshwater cladoceran, *Daphnia magna.*

P = freshwater algae *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum.*

T = rainbow trout, *Oncorhynchus mykiss* formerly called *Salmo gairdneri.*

S = sheepshead minnow, *Cyprinodon variegatus.*

R = algae growth rate.

B = algae biomass.

TABLE 6. EVALUATION OF AQUATIV TOXICOLOGY OF PETROLEUM ADDITIVE ALKARYL SULFONATES (CONT.)

CAS Number	ACUTE TOXICITY TO FISH 96-hr LL_{50} (mg/L)	ACUTE TOXICITY TO INVERTEBRATES 48-hr EL_{50} (mg/L)[1]	TOXICITY TO ALGAE 96-hr EL_{50} (mg/L)
	Available Data & Proposed Testing	Available Data & Proposed Testing	Available Data & Proposed Testing
71549-79-6	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
71486-79-5	>1,000 (WAF[2], F)	>1,000 (WAF', D)	>1,000 (WAF[3], P, R) >1,000 (WAF[3], P, B)
70024-69-0	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
70024-71-4 C20-C24 analog of 70024-69-O	>10,000 (WAF[2], S)	No testing needed	No testing needed
71786-47-5	>1,000 (WAF[2], F) >10,000 (WAF[2], S)	>1,000 (WAF', D)	>1,000 (WAF', P, R) >1,000 (WAF[3], P, B)

'Toxicity endpoints are expressed as median lethal loading rates (LL_{50}) for fish and median effective loading rates (EL_{50}) for *Daphnia* and algae. The EL/LL_{50} is defined as the loading rate that adversely effects 50% of the test organisms exposed to it during a specific time. The greater the EL/LL_{50} the lower the toxicity.

[2]WAF = Water accommodated fraction static renewal test.

[3]WAF = Water accommodated fraction static non-renewal test

[4]EL/LL_0 = no mortality or effects observed at the highest loading rate tested.

F = fathead minnow, *Pimephalespromelas.*

D = freshwater cladoceran, *Daphnia magna.*

P = freshwater algae *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum.*

T = rainbow trout, *Oncorhynchus mykiss* formerly called *Salmo gairdneri.*

S = sheepshead minnow, *Cyprinodon variegatus.*

R = algae growth rate.

B = algae biomass.

TABLE 7. EVALUATION OF ACUTE MAMMALIAN TOXICOLOGY OF PETROLEUM ADDITIVE ALKARYL SULFONATES

CAS Number	ACUTE ORAL TOXICITY' Available Data & Proposed Testing	ACUTE DERMAL TOXICITY' Available Data & Proposed Testing	ACUTE INHALATION TOXICITY' Available Data & Proposed Testing
115829-3 6-2	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
115733-09-O	LD_{50} > 5.0 g/kg (rat)	LD_{50} >5.0 g/kg (rabbit)	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
115733-10-3	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
68608-26-4	LD_{50} > 5.0 g/kg (rat)	No testing needed Acute toxicity end point satisfied by acute oral toxicity results	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
6 1789-86-4	LD_{50} > 5.0 g/kg (rat)	LD_{50} > 5.0 g/kg (rabbit)	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
68783-96-O	LD_{50} > 5.0 g/kg (rat)	LD_{50} > 2.0 g/kg (rabbit)	[2]LC_0 = 1.9 mg/L (rat)
61790-48-5	LD_{50} > 2.0 g/kg (rat)	No testing needed Acute toxicity end point satisfied by acute oral toxicity results	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
78330-12-8	LD_{50} > 5.0 g/kg (rat)	No testing needed Acute toxicity end point satisfied by acute oral toxicity results	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
7 1549-79-6	LD_{50} = 14.9g/kg (rat)	No testing needed Acute toxicity end point satisfied by acute oral toxicity results	No testing needed Acute toxicity end point satisfied by acute oral toxicity results

[1]Toxicity endpoints are expressed as median lethal dose (LD_{50}) for acute oral and dermal toxicity and median lethal concentration (LC_{50}) for acute inhalation toxicity. The LD/LC_{50} is defined as the dose/concentration that is lethal to 50% of the test organisms. The greater the LD/LC_{50}, the lower the toxicity.
[2]LC_0 = no mortality observed at the highest concentration tested.

TABLE 7. EVALUATION OF ACUTE MAMMALIAN TOXICOLOGY OF PETROLEUM ADDITIVE ALKARYL SULFONATES (CONT.)

CAS Number	ACUTE ORAL TOXICITY' Available Data & Proposed Testing	ACUTE DERMAL TOXICITY' Available Data & Proposed Testing	ACUTE INHALATION TOXICITY' Available Data & Proposed Testing
71486-79-8	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
-70024-69-o	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
C20-C24 alkaryl calcium salt (no CAS #) analog of 70024-69-o	LD_{50} > 5.0 g/kg (rat)	LD_{50} > 5.0 g/kg (rat)	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
7 178647-5	LD_{50} > 16.0 g/kg (rat)	No testing needed Acute toxicity end point satisfied by acute oral toxicity results	No testing needed Acute toxicity end point satisfied by acute oral toxicity results

'Toxicity endpo :s are expressed as median lethal dose (LD_{50}) for acute oral and dermal toxicity and median lethal concentration (LC_{50}) for acute inhalation toxicity. The LD/LC_{50} is defined as the dose/concentration that is lethal to 50% of the test organisms. The greater the LD/LC_{50}, the lower the toxicity.
'LC_0 = no mortality observed at the highest concentration tested.

TABLE 8. EVALUATION OF MUTAGENICITY OF PETROLEUM ADDITIVE ALKARYL SULFONATES

CAS Number	GENE MUTATION ASSAY Available Data & Proposed Testing	CHROMOSOMAL ABERRATION ASSAY Available Data & Proposed Testing
115829-3 6-2	No testing needed Bridging	No testing needed Bridging
115733-09-O	No testing needed Bridging	No testing needed Bridging
115733-l o-3	No testing needed Bridging	No testing needed Bridging
68608-26-4	No testing needed Bridging	No testing needed Bridging
6 1789-86-4	No testing needed Bridging	No testing needed Bridging
68783-96-O	Bacterial Reverse Mutation Assay – Not mutagenic Mouse Lymphoma Mutagenicity Screen Not mutagenic	Mouse Micronucleus Assay – Not clastogenic In *vitro* CHO Cell Chromosomal Aberration Assay – Not clastogenic
6 1790-48-5	No testing needed Bridging	No testing needed Bridging
C15-C21 alkaryl sodium salt (no CAS #) analog of 78330-12-8	Bacterial Reverse Mutation Assay Not mutagenic	No testing needed Bridging
78330-12-g	No testing needed Bridging	No testing needed Bridging
7 1549-79-6	No testing needed Bridging	No testing needed Bridging
7 1486-79-8	No testing needed Bridging	No testing needed Bridging

TABLE 8. EVALUATION OF MUTAGENICITY OF PETROLEUM ADDITIVE ALKARYL SULFONATES (CONT.)

CAS Number	GENE MUTATION ASSAY	CHROMOSOMAL ABERRATION ASSAY
	Available Data & Proposed Testing	**Available Data & Proposed Testing**
70024-69-O	No testing needed Bridging	No testing needed Bridging
C20-C24 alkaryl calcium salt (no CAS #) analog of 70024-69-o	Bacterial Reverse Mutation Assay – Not mutagenic	Mouse Micronucleus Assay – Not clastogenic
7 1786-47-5	Bacterial Reverse Mutation Assay – Not mutagenic	Mouse Micronucleus Assay – Not clastogenic *In vitro* CHO Cell Chromosomal Aberration Assay – Not clastogenic

TABLE 9. EVALUATION OF REPEATED-DOSE MAMMALIAN TOXICOLOGY OF PETROLEUM ADDITIVE ALKARYL SULFONATES

CAS Number	REPEATED-DOSE TOXICITY Available Data & Proposed Testing	REPRODUCTIVE/DEVELOPMENTAL TOXICITY Available Data & Proposed Testing
115829-36-2	No testing needed Bridging	No testing needed Bridging
115733-09-O	Test	Test
115733-10-3	No testing needed Bridging	No testing needed Bridging
68608-26-4	No testing needed Bridging	No testing needed Bridging
6 1789-86-4	No testing needed Bridging	No testing needed Bridging
68783-96-O	**2%day repeated-dose dermal study in rats (OECD 410)** NOAEL = 1000 mg/kg/day (highest dose tested) **28-day inhalation study in rats (OECD 412)** NOAEL = 49.5 mg/m^3 At 260 mg/m^3, • signs of toxicity, • decreased body weight gain (males), • increased lung weights, • intraalveolar microphage accumulation, • bronchiole epithelium hyperplasia/hypertrophy; At 156 mg/m^3, • signs of toxicity, . increased lung weights, • intra-alveolar microphage accumulation, • bronchiole epithelium hyperplasia/hypertrophy; At 49.5 mg/m^3, • no significant effects.	No testing needed Bridging

TABLE 9. EVALUATION OF REPEATED-DOSE MAMMALIAN TOXICOLOGY OF PETROLEUM ADDITIVE ALKARYL SULFONATES (CONT.)

CAS Number	REPEATED-DOSE TOXICITY Available Data & Proposed Testing	REPRODUCTIVE/DEVELOPMENTAL TOXICITY Available Data & Proposed Testing
61790-48-5	No testing needed Bridging	No testing needed Bridging
78330-12-g	No testing needed Bridging	No testing needed Bridging
7 1549-79-6	No testing needed Bridging	No testing needed Bridging
71486-79-8	No testing needed Bridging	No testing needed Bridging
70024-69-o	No testing needed Bridging	No testing needed Bridging
C20-C24 alkaryl calcium salt (no CAS #) analog of 70024-69-O	**4-week repeated-dose oral study in rats (OECD 407)** NOAEL = 500 mg/kg/day At 1000 mg/kg/day, • decreased serum cholesterol; At 500 mg/kg/day, • no significant effects; At 100 mg/kg/day, • no significant effects.	No testing needed Bridging

TABLE 9. EVALUATION OF REPEATED-DOSE MAMMALIAN TOXICOLOGY OF PETROLEUM ADDITIVE ALKARYL SULFONATES (CONT.)

CAS Number	REPEATED-DOSE TOXICITY Available Data & Proposed Testing	REPRODUCTIVE/DEVELOPMENTAL TOXICITY Available Data & Proposed Testing
71786-47-5	**28-day repeated dose dermal study in rats (OECD 410)** NOAEL = 1000 mg/kg/day (highest dose tested). **2%day repeated dose dermal study in rabbits (OECD 410)** At 2.0 ml/kg/day, · two males sacrificed in moribund condition; · decreased mean body weight; · alopecia and erythema, edema, atonia, desquamation, fissuring, and exfoliation of the skin; · decreased total leukocyte count: · decreased red blood cell count, hemoglobin, and hematocrit,(females only); · decreased total serum protein and serum globulin; · increased SGOT and serum alkaline phosphatase (males); · increased SGOT and SGPT (females); · increased liver weights and focal hepatocellular degeneration, necrosis, and vacuolation; · decreased testes weights with aspermatogenesis, decreased number of spermatids and diffuse tubular hypoplasia; • decreased epididymides weights with epithelial hypoplasia; At 0.5 ml/kg/day, · alopecia and erythema, edema, atonia, desquamation, fissuring, and exfoliation of the skin; · decreased total leukocyte count; · decreased total serum protein and serum globulin; · increased SGOT and serum alkaline phosphatase (males); · increased SGOT and SGPT (females); · increased liver weights; • decreased testes and epididymides weights.	No testing needed Bridging

AR201-13206B

Substance Group: **Group 3: Alkaryl Sulfonates**

Summary prepared by: **Petroleum Additives Panel**
Health & Environmental Research Task Group
October 9, 2001

2.0 Melting and Boiling Points

CAS #	Parameter	Value*
61789-86-4	°C melting point	349.84
	°C boiling point	935.88
61790-48-5	°C melting point	349.84
	°C boiling point	935.88
68608-26-4	°C melting point	309.31
	°C boiling point	707.03
68783-96-0	°C melting point	349.84
	°C boiling point	935.88
Analog of 70024-69-0	°C melting point	349.84
	°C boiling point	935.88
71549-79-6	°C melting point	208.45
	°C boiling point	506.34
71786-47-5	°C melting point	349.84
	°C boiling point	935.88
78330-12-8	°C melting point	347.25
	°C boiling point	788.26
115733-09-0	°C melting point	349.84
	°C boiling point	935.88
115829-36-2	°C melting point	208.45
	°C boiling point	506.34

*These data were modeled by an HERTG member company representative. The reliability code that should accompany the robust summaries prepared using these data is: (2) Valid with restrictions. The selection of this code is based on the data being modeled rather than measured. The use of these data should always be accompanied by the caveat that they were modeled using a structure based modeling program (see reference) and that the values selected are based on a structure that is representative of the CAS#.

The reference for the model is:
MPBPWIN (v1.31) In: Meylan W. and P. Howard. 1999. EPIWIN Modeling Program, Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY, 13212-2510, USA.

3.0 Biodegradation

Robust Summary 3-Biodeg-1

Test Substance	
CAS #	61789-86-4
Chemical Name	Sulfonic acids, petroleum, calcium salts
Remarks	This substance is referred to as petroleum derived calc ium salt in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	OECD 301F
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1996
Contact time (units)	28 days.
Inoculum	Activated sludge from domestic wastewater treatment plant
Remarks for test conditions	Inoculum: The supernatant from the homogenized activated sludge was used as inoculum. The inoculum was pre-adapted to the test material for 14 days during which the test substance was added incrementally at concentrations equivalent to 4, 8, and 8 mg carbon/L on days 0, 7, and 12, respectively. The targeted microbial level in the test mixture was 10,000 to 1,000,000 cells/mL. Concentration of test chemical: Test substance concentration was approximately 100 mg/L, giving at least 50 to 100 mg ThOD per L test medium. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a teflon coupon and introduced into the medium. Temp of incubation: 23 ± 1°C Dosing procedure: A measured volume of the inoculated mineral medium containing approximately 100 mg/L test substance was continuously stirred in a closed system for 28 days. Sampling frequency: The oxygen uptake was monitored continuously and recorded every 4 hours throughout the test.

Robust Summary 3- Biodeg-2

Test Substance	
CAS #	Analog of 7 1786-47-5
Chemical Name	Magnesium long chain alkaryl sulfonate
Remarks	This substance is an analog for the group of substances referred to as alkaryl magnesium salt derivative, in HERTG's Test Plan for the Alkaryl Sulfonates Category. It is chemically similar to members of this CAS number, but does not belong to this CAS number. For more information on these substances, see Section 2.0 "Chemical Description of Alkaryl Sulfonate Category" in HERTG's Test Plan for the Alkaryl Sulfonate Category.
Method	
Method/Guideline Followed	OECD 301B, Ready Biodegradability, Modified Sturm Test
Test Type (aerobic/anaerobic j	Aerobic
GLP (Y/N)	Y
Year (study performed)	1995
Contact time (units)	28 days
Inoculum (source)	Domestic activated sewage sludge
Remarks For Test Conditions	Inoculum: Activated sewage sludge from domestic WWTP prepared per test guideline. Inoculum was not acclimated. Replicates: Triplicates for test substance, positive control material, and control blank. Temperature of incubation: 20 23 °C Dosing procedure: Neat test chemical was gravametrically determined on glass cover slips, which were then added to culture medium in test vessels. Sampling: Days 2, 4, 7, 10, 14, 17, 21, 24, 29 (after acidification on day 28) Concentration of test substance: Loading into each of 3 test vessels were 19.9, 20.1, and 20.0 mg C/L. Controls: Blank and positive controls used per guideline; toxicity control not used Positive control was benzoic acid (Na salt) added to each control vessel at a loading of 20.2 mg C/L. Analytical method: Titration of residual Ba(OH)2 (0.05 N initially) in trapping solution, using O.lN HCl. Method of calculating biodegradation values: Percent biodegradation calculated as percent ratio of cumulative net carbon dioxide to theoretical carbon dioxide as determined from elemental analysis of test material.

Results	
Degradation % After Time	Test substance: 1.5% in 28 days Positive control substance: 89.2% in 28 days
Kinetics (for sample, positive and negative controls)	Positive control $t_{1/2}$: <10 days
Breakdown Products (Y/N) (if yes describe breakdown products)	N
Conclusions	1.5% in 28 days
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 9-6-00

RobustSummary 3- Biodeg-3

Test Substance	
CAS #	Analog of 70024-69-o
Chemical Name	Benzenesulfonic acid, mono-C16-C24-alkyl derivatives, calcium salts
Remarks	This substance is referred to as C16-C24 alkaryl calcium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Closed bottle test according to OECD Guideline No. 301D, EEC Directive 79/831 and EEC Directive 67/548 Annex V C.6 as published in 84/499/EEC.
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1989
Contact time (units)	28 days
Inoculum	Domestic activated sewage sludge
Remarks for test conditions	Inoculum: Activated sludge bacteria from domestic sewage treatment plant used at about 1 drop sludge filtrate inoculum/L basal medium. Concentration of test chemical: 2 mg/L. Inoculum was not pre-acclimated to test substance. Two replicates run per treatment. Temperature of incubation: 20±1°C Dosing procedure: Test chemical was added onto Whatman GFA filter paper that were then placed inside test vessels immediately before culture medium was added to the vessels. Sampling: Days 0, 5, 15, and 28 after inoculation. Controls: Yes (blank and positive controls used per guideline); toxicity control not used. Standard Control Substances: Sodium benzoate and Aniline tested at 2 mg/L. Analytical method: Chemical oxygen demand (COD) of the test substance and standard control substances determined using the Hach semi-micro sample digestion methods followed by direct reading of the CODs using a Hach DR/2 Spectrophotometer. During the biodegradability test dissolved oxygen concentrations for each test medium were determined in duplicate using a Yellow Springs BOD Probe.

Remarks for test conditions, cont'd	Inoculum: Sludge from domestic WWTP used at 10 mL/L basal medium Conc of test chemical: Test chemical added directly to test vessels at 13.3 mg C/L (28.6 mg/L CAS# 685 l l-50-2). No preacclimation was used. Temp of incubation: 23 – 24 °C Dosing procedure: Neat test chemical added by micropipettor to culture medium in vessels immediately prior to addition of sewage and soil inocula Sampling: Days 1, 3, 6, IO. 14, 21, 29 (after acidification on d 28) Controls: Yes (blank and positive controls used per guideline); toxicity control not used. Positive Control was Benzoic acid (Na salt) at 20 mg C/L Analytical method: Titration of residual Ba(OH)2 in trapping solution, using HCl Method of calculating measured concentrations: The oxygen depletion values for the test substance and standard substances at each sampling time are corrected by means of the blank values and expressed as a percentage of the theoretical oxygen demand or chemical oxygen demand determined by the Hach semi-micro sample digestion method. Other: • Biodegradation of the Standard Control Substances: Sodium benzoate and Aniline attained 97% and 61% degradation within 28 days. Because both standard substances achieved greater or equal to 60% degradation the test was deemed valid. • Two replicates were run per treatment; values are average of replicates. The % biodegradation value reported is slightly inflated by the use of zero titration volume rather than negative volume when corrected for blanks; however, comparison of titration volumes for the test chemical and blank show them to be very similar, so inhibition of inoculum is not suspected.
Results	
Degradation % after time	Test Substance degraded 8.0% by day 28.
Kinetic (for sample, positive and negative controls)	None given
Breakdown Products (Y/N) If yes describe breakdown products	NA

Conclusions	8.0% Not Readily biodegradable; biodegradation was essentially zero
Data Quality	(1) Reliable without restriction
References	Douglas, M.T. (1989) The Ready Biodegradability of Analog of CAS# 70024-69-o in a Closed bottle Test System, Huntington Research Centre, Ltd., Study #30/891706.
Other	Updated: 9-6-00

Robust Summarv 3- Biodeg 4

Test Substance	
CAS #	Analog for 68783-96-O
Chemical Name	Calcium alkaryl sulfonate
Remarks	This substance is an analog for the group of substances referred to as petroleum derived calcium salt, overbased, in HERTG's Test Plan for the Alkaryl Sulfonates Category. It is chemically similar to members of this CAS number, but does not belong to this CAS number. For more information on these substances, see Section 2.0 "Chemical Description of Alkaryl Sulfonate Category" in HERTG's Test Plan for the Alkaryl Sulfonate Category.
Method	
Method/Guideline Followed	OECD 3018, Ready Biodegradability, Modified Sturm Test
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (study performed)	1995
Contact time (units)	28 davs
Inoculum (source)	Domestic activated sewage sludge
Remarks For Test Conditions	Inoculum: Activated sewage sludge from domestic WWTP prepared per test guideline. lnoculum was not acclimated. Replicates: Triplicates for test substance, positive control material, and control blank. Temperature of incubation: 20 – 23 °C Dosing procedure: Neat test chemical was gravametrically determined on glass cover slips, which were then added to culture medium in test vessels. Sampling: Days 1, 3, 5, 7, 10, 13, 17, 20, 24, 29 (after acidification on day 28) Concentration of test substance: Loadings into 3 test vessels were 19.8, 20.1, and 19.4 mg C/L. Controls: Blank and positive controls used per guideline; toxicity control not used. Positive control was benzoic acid (Na salt) added to each control vessel at a loading of 21.2 mg C/L. Analytical method: Titration of residual Ba(OH)2 (0.05 N initially) in trapping solution. using 0.1N HCl. Method of calculating biodegradation values: Percent biodegradation calculated as percent ratio of cumulative net carbon dioxide to theoretical carbon dioxide as determined from elemental analysis of test material.

Results	
Degradation % After Time	Test substance: 9.1% in 28 days Positive control substance: 86.1% in 28 days
Kinetics (for sample, positive and negative controls)	Positive control $t_{1/2}$: <10 days
Breakdown Products (YIN) (if yes describe breakdown products)	N
Conclusions	***9.1%*** in 28 days
Data Quality	(1) Reliable without restriction
References	This robust summary was prepa red from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information .
Other	Updated: 9-6-00

AQUATIC ORGANISMS

4.1 Acute and Prolonged Toxicity to Fish

Robust Summary 3-Fish Tox -1

Test Substance	
CAS #	**6** 1789-86-4
Chemical Name	Sulfonic acids, petroleum, Calcium salts
Remarks	This substance is referred to as petroleum derived calcium salt in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	Static renewal test; a one level screening test
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Sheepshead minnow (*Cyprinodon variegatus*)
Analytical Monitoring	Total organic carbon (TOC) measurements of each freshly prepared test solution and control and after 24-h on test just before daily renewal with fresh test solution.
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in the study.
Remarks field for test conditions (fill as applicable)	Test Organisms: source – a commercial supplier in New Hampshire, age – 14 days old, total length 12 mm average (range 10 to 15 mm; n =30), wet weight – 0.040 g average (range 0.004 to 0.11 g; n = 30). Loading – 0.080 g biomass/L, Pretreatment none, fish held for a minimum of 7 days before testing. No feeding during the test. Test System: Individual WAFs (individual water accommodated fractions) were prepared for each daily renewal of the 10,000 mg/L test level. A measured weight of test material was added to a measured volume of dilution water (15-L) in a glass vessel and stirred for 16 to 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the

	mixing period, the test solution was allowed to stand for 2 hours before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end approximately midway between bottom and surface. The siphoned water phase, designated WAF, was used for the aquatic toxicity test. About 90% of the test solution in each test vessel was renewed daily after 24, 48, and 72 hours. Two 5-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels were loosely covered to reduce entry of dust. Dilution Water: Natural seawater collected from Cape Cod Canal, Bourne, Massachusetts. The water was filtered through 0.5-micron polypropylene core filter and activated carbon, then stored for 1 to 4 days prior to use while being constantly aerated. During storage the water had a salinity of 32 to 34 ppt and pH of 7.7 to 7.8. During the test: dissolved oxygen – 6.3 mg/L to above 100% saturation (7.2 mg/L), pH – 7.X to 8.1, salinity – 32 to 34, temperature – 22 to 23 C. Mean measured TOC levels in the control and 10,000 mg/L WAF test level were 4.1 mg/L (range 2.3 to 7.9) and 10.2 mg/L (range 6.4 to 15.0), respectively Test Levels: Control & 10,000 mg/L WAF loading rate. Test Findings: No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test. Calculation of LL_{50} s: Statistical analysis of survival data not warranted. Test Substance: No undissolved test material was report on the surface of the test vessels during the entire aquatic toxicity test. Reference Substance: Sodium lauryl sulfate (SLS). The 96-h LC_{50} was 1.2 mg/L. No information provided on method of calculation.
Results	Nominal concentrations: 96-h LL_{50} > 10,000 mg/L. This is equivalent to 96-h LL_0 = 10,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Statistical analysis of survival data not warranted. Other:

	• Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Nicholson. R.B. (1986) Acute toxicity of CMA Test Material Code 504 to Sheepshead Minnow, *Cyprinodon variegatus.* Springborn Bionomics Study #10823-0186-6 100-500-504, Report #BW-86-04-*1983.*
Other	Updated: o-6-00

Robust Summary 3-Fish Tox:2

Test Substance	
CAS #	analog for Analog of CAS # 70024-69-o (material tested = CAS #70024-7 l-4)
Chemical Name	analog to benzenesulfonic acid, mono-C 1 6–C24 alkyl derivatives, calcium salts, overbased
Remarks	The tested substance is an analog for the group of substances referred to as Cl 6-C24 alkyl calcium salt overbased derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. It is chemically similar to members of this CAS number, but does not belong to this CAS number. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	Static renewal test; a one level screening test
GLP (Y/N)	Y
Year (Study Performed)	1986
Species&train	Sheepshead minnow (*Cyprinodon variegatus*)
Analytical Monitoring	Total organic carbon (TOC) measurements of each freshly prepared test solution and control and after 24-h on **test just** before daily renewal with fresh test solution.
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in the study.
Remarks field for test conditions (fill as applicable)	Test Organisms: source – a commercial supplier in New Hampshire, age – 10 to 15 days old, total length – 11 mm average (range 10 to 13 mm; n =30), wet weight – 0.028 g average (range 0.02 to 0.05 g; n = 30). Loading – 0.056 g biomass/L, Pretreatment – none, fish held for a minimum of 6 days before testing. No feeding during the test. Test System: Individual water accommodated fractions (WAFs) were prepared for each daily renewal of the 10,000 mg/L test level. A measured weight of test material was added to a measured volume of dilution water (15-L) in a glass vessel and stirred for 16 to 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solution were allowed to stand for 2 hours before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end approximately midway between bottom

	and surface. The siphoned water phase, designated water accomodated fraction (WAF), was used for the aquatic toxicity test. About 90% of the test solution in each test vessel was renewed daily after 24, 48, and 72 hours. Two 5-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels were loosely covered to reduce entry of dust. Dilution Water: Natural seawater collected from Cape Cod Canal, Boume, Massachusetts. The water was filtered through 0.5-micron polypropylene core filter and activated carbon, then stored for 1 to 4 days prior to use while being constantly aerated. During storage the water had a salinity of 32 to 34 ppt and pH of 7.7 to 7.8. During the test: dissolved oxygen – 5.5 mg/L to above 100% saturation (7.2 mg/L), pH – 7.9 to 8.1, salinity – 32 to 34, temperature – 22 to 23 C. Mean measured TOC levels in the control and 10,000 mg/L WAF test level were 3.0 mg/L (range 1.2 to 4.4) and 6.4 mg/L (range 5.0 to 7.9), respectively. Test Levels: Control & 10,000 mg/L WAF loading rate. Test Findings: No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test. Calculation of LL_{50} s: Statistical analysis of survival data not warranted because there was no mortality in the study. Test Substance: No undissolved test material was report on the surface of the test vessels during the entire aquatic toxicity test. Reference Substance: Sodium lauryl sulfate (SLS). The 96-h LC_{50} was 1.2 mg/L. No information provided on method of calculation.
Results	Nominal concentrations: 96-h LL_{50} >10,000 mg/L. This is equivalent to 96-h LL_0 = 10,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Statistical analysis of survival data not warranted. Other: • Test results reported in original study as “lethal concentrations” are reported in this summary as “lethal loading”, because test results are based on WAF loading rates. • Control response was satisfactory.

Conclusions	No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Nicholson, R.B. (1986) Acute toxicity of CMA Test Material Code 5 14 to Sheepshead Minnow, *Cyprinodon variegatus.* Springborn Bionomics Study #10823-0186-6100-500-514, Report #BW-86-04-*1993.*
Other	Updated: 9-6-00

Robust Summary 3-Fish Tox:3

Test Substance	
CAS #	7 1486-79-8
Chemical Name	Benzenesulfonic acid, mono-C 15-3 O-branched alkyl and di-C 1 l-13-branched and linear alkyl derivatives, calcium salts, overbased
Remarks	This substance is referred to as mixed Cl 5-C30 and C I l- 13 alkaryl calcium salt, overbased derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1400 (1985), OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	Static renewal test
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain	Fathead minnow *(Pimephalespvomelas)*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (C-h) test solutions and after one day on test (24-h) before renewal of fresh test solutions. Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in the study.
Remarks field for test conditions (fill as applicable)	Test Organisms: Acquired from Aquatic Research Organisms, Hampton, New Hampshire, age: juvenile, total length: 29 mm average (longest fish not more than twice the shortest fish), wet weight: 0.2 g average (no range reported). Loading: <0.5 g biomass/L, Pretreatment: none, fish held for a minimum of 14 days before testing. No feeding during the test. Test System: Individual water accommodated fractions (WAFs) were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (30-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end l-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used in the aquatic toxicity test. About 80% of the solution

	in each test level was renewed daily after 24, 48, and 72 hours. Two 15-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels loosely covered to reduce entry of dust. Dilution Water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness of 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer, and then it was stored in a polyethylene tank where it was aerated. The water was characterized as moderately hard water. Light: 16-h light per day using cool-white fluorescent lights with an intensity of 20 uEin/m^2. Test Temperature: 2 1.4 to 22.8 C. Water Chemistry: Dissolved oxygen: 7.3 – 8.6 mg/L, pH: 7.4 - 8.1, conductivity: 860 – 910 umhos/cm. Alkalinity not reported. Element: Mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Test Findings: No mortality or signs of toxicity was observed in all treatments and the control throughout the entire test. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Analytical Monitoring: TOC levels were between 2.3 - 3.0 mg/L in the control, 2.X - 3.2 mg/L at 100 mg/L loading, between 2.6 - 3.2 mg/L at 300 mg/L loading and 2.6 - 3.3 mg/L at the 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Reference Substance: No
Results	Nominal concentrations: 96-h LL_{50} >1,000 mg/L. This is equivalent to 96-h LL_0 = 1,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L

	LC50, LCO, LL50 or LLO at 48, 72, 96-hours: LL_{50} and LL_0 reported as LC_{50} and NOEC, respectively, although test results were based on WAF loading rate. Statistical results: Statistical analysis of survival data not warranted. Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates, • Control response was satisfactory.
Conclusions	No mortality or signs of toxicity were observed in any of the treatments (100, 300, and 1,000 mg/L WAF loading rates) or in the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of The Water Accommodated Fractions (WAFs) of CMA 60.5 to The Fathead Minnow, *Pimephales promelas.* T.R. Wilbury Study #9176-CMA/ESI-605.
Other	Updated: 9-6-00

Robust Summary 3-Fish Tox:4

Test Substance	
CAS #	7 1786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts
Remarks	This substance is referred to as alkaryl magnesium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1400 (1985), OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	Static renewal test
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain	Fathead minnow (*Pimephales promelas*)
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (O-h) test solutions and after one day on test (24-h) before renewal of fresh test solutions. Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 4 15.1
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in this study.
Remarks field for test conditions (fill as applicable)	Test Organisms: Acquired from Aquatic Research Organisms, Hampton. New Hampshire, age: juvenile, total length: 38.4 mm average (longest fish not more than twice the shortest fish), wet weight: 0.5 g average (no range reported). Loading: <0.5 g biomass/L, Pretreatment: none, fish held for a minimum of 14 days before testing. No feeding during the test. Test System: Individual water accommodated fractions (WAFs) were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (30-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for I hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end l-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used in the aquatic toxicity test. About 80% of the solution in each test level was renewed daily after 24, 48, and 72 hours. Two

	15-L replicates per treatment, 10 fish per replicate (20 per treatment), Test vessels loosely covered to reduce entry of dust. Dilution Water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness of 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer, and then it was stored in a polyethylene tank where it was aerated. The water was characterized as moderately hard water. Light: 16-h light per day using coo!-white fluorescent lights with an intensity of 20 uEin/m^2. Test Temperature: 2 1.6 to 22.8 C. Water Chemistry: Dissolved oxygen: 6.9 - 8.3 mg/L, pH: 7.0 - 7.9, conductivity: 870 890 umhos/cm. Alkalinity not reported. Element: Mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Test Findings: No mortality or signs of toxicity was observed in all treatments and the control throughout the entire test. Calculation of LL_{50} s: Statistical analysis of survival data not warranted. Analytical Monitoring: TOC levels were between 2.8 - 3.2 mg/L in the control, 3.3 - 3.8 mg/L at 100 mg/L loading, between 3.1 - 4.0 mg/L at 300 mg/L loading and 3.2 - 4.4 mg/L at the 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Reference Substance: No
Results	Nominal concentrations: 96-h LL_{50} >1,000 mg/L. This is equivalent to 96-h LL_0 = 1,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L

	Statistical results: Statistical analysis of survival data not warranted. Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	No mortality or signs of toxicity were observed in any of the treatments (100, 300, and 1,000 mg/L WAF loading rates) or in the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of The Water Accommodated Fractions (WAFs) of CMA 609 to The Fathead Minnow, *Pimephales promelas.* T.R. Wilbur-y Study #9176-CMA/ESI-609.
Other	Updated: 9-6-00

Robust Summary 3-Fish Tox: 5

Test Substance	
CAS #	7 1786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, Magnesium salts
Remarks	This substance is referred to as alkaryl magnesium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	static renewal test; a one level screening test
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Sheepshead minnow (*Cyprinodon variegatus*)
Analytical Monitoring	Total organic carbon (TOC) measurements of each freshly prepared test solution and control and after 24-h on test just before daily renewal with fresh test solution.
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in this study.
Remarks field for test conditions (fill as applicable)	Test Organisms: source a commercial supplier in New Hampshire, age – 17 to 22 days old, tota 1 length – 11 mm average (range 10 to 13 mm; n =30), wet weight 0.028 g average (range 0.02 to 0.05 g; n = 30). Loading - 0.056 g biomass/L, Pretreatment – none, fish held for a minimum of 20 days before testing. No feeding during the test. Test System: Individual water accomodated fractions (WAFs) were prepared for each daily renewal of the 10,000 mg/L test level. A measured weight of test material was added to a measured volume of dilution water (15-L) in a glass vessel and stirred for 16 to 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solution were allowed to stand for 2 hours before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end approximately midway between bottom and surface. The siphoned water phase, designated WAF was used for the aquatic toxicity test. About 90% of the test solution in each test vessel was renewed daily after 24, 48, and 72 hours. Two 5-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels were loosely covered to reduce entry of dust.

	Dilution Water: Natural seawater collected from Cape Cod Canal, Bourne, Massachusetts. The water was filtered through 0.5-micron polypropylene core filter and activated carbon, then stored for 1 to 4 days prior to use while being constantly aerated. During storage the water had a salinity of 32 to 34 ppt and pH of 7.7 to 7.8. During the test: dissolved oxygen -- 4.9 mg/L to above 100% saturation (7.4 mg/L), pH – 7.6 to X.1, salinity – 32 ppt, temperature – 22 to 23 C. Mean measured TOC levels in the control and 1,000 mg/L WAF test level were 5.0 mg/L (range 2.6 to 7.0) and 3.6 mg/L (range 1.0 to 7.5), respectively. Test Levels: Control & 10,000 mg/L WAF loading rate. Test Findings: No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test. Analytical Monitoring:). Mean measured TOC in the 10,000 mg/L WAF test level was 5.0 mg/L compared to 3.6 mg/L in the control. Test Substance: No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Reference Substance: Sodium lauryl sulfate (SLS). The 96-h LC_{50} was 1.2 mg/L. No information provided on method of calculation.
Results	Nominal concentrations: 96-h LL_{50} > 10,000 mg/L. This is equivalent to 96-h LL_0 = 10,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Statistical analysis of survival data not warranted because there was no mortality in this study. Other: • Test results reported in original study as “lethal concentrations” are reported in this summary as “lethal loading”, because test results are based on WAF loading rates. • Control response was satisfactory.

Conclusions	No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Nicholson, R.B. (1986) Acute toxicity of CMA Test Material Code 523 to Sheepshead Minnow, *Cyprinodon variegatus.* Springbom Bionomics Study #10823-0186-6 100-500-523, Report #BW-86-04-1986.
Other	Updated: 9-6-00

4.2 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

Robust Summary 3-Aquatic Invertebrates Tox - 1

Test Substance	
CAS #	115733-09-0
Chemical Name	Benzenesulfonic acid, C 14-C24 branched and linear alkyl derivatives, calcium salts
Remarks	This substance is referred to as C 14-C24 alkyl calcium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1985, 1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1993
Species/Strain	Cladoceran, *Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (O-h) test solutions and at test termination (48-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1.
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis of data not warranted.
Remarks field for test conditions (fill as applicable)	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end l-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.

	Test conditions: Two 250-mL glass beakers that contained 200 mL of test solution were used per treatment. The 250-mL test vessels were loosely covered to reduce entry of dust. Light: 16-hour light per day using cool-white fluorescent lights with an intensity of 20 uEin/m^2. Test temperature: 20.4 – 20.9 C Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer and stored in a polyethylene tank where it was aerated. TOC levels were 2 mg/L at the beginning and end of the test and <10 mg/L at the end of the test. Water chemistry: Dissolved oxygen: 7.9 - 8.7 mg/L; pH: 7.2 - 8.1; conductivity: X60 – 880 umhos/cm. Element: Immobilization/mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates: 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test. Test Findings: At 24 hours, no immobilized or dead organisms were observed in the control or treatments. At 4%hours 5, 0, 20, and 5% immobilization were reported for control, 100, 300, and 1,000 mg/L, respectively. Calculation of EL_{50} s: Statistical analysis of survival data not warranted. Exposure period: 48 hours Analytical Monitoring: TOC levels were 2.8 – 3.5 mg/L in the control, 2.8 - 3.6 mg/l at 100, 3.0 - 3.7 mg/L at 300 mg/L, and 2.7 - 3.4 mg/L at 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	Nominal concentrations: 48-h EL_{50} >1,000 mg/L. This is equivalent to 48-h EL_0 = 1000 mg/L based on WAF loading rates.
Remarks	Measured concentration: n/a

	Unit: mg/L Statistical results: Not applicable. Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • 20% immobilization/mortality seen at the middle test concentration of 300 mg/L (WAF) was not considered to be treatment related. This was based on findings of insignificant effects, or no effects, in both the highest and lowest test levels, respectively. • Control response was satisfactory.
Conclusions	The test material was not toxic to daphnids at loading rates tested. Percent survival/unaffected organisms was 95% in the control, 100% at 100, 80% at 300, and 95% at 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #604 to the Daphnid, *Daphnia* magna. T.R. Wilbury Study #9178-CMA/ESI-604.
Other	Updated: 9-6-00

Robust Summary 3-Aquatic Invertebrate Tox-2

Test Substance	
CAS #	71486-79-8
Chemical Name	Benzenesulfonic acid, mono-C15-30-branched alkyl and di-C11-13-branched and linear alkyl derivatives, calcium salts, overbased
Remarks	This substance is referred to as mixed C15-C30 and C11-13 alkaryl calcium salt, overbased derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1985, 1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1993
Species/Strain	Cladoceran, *Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and at test termination (48-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1.
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis of data not warranted because immobilization seen at high dose not significant.
Remarks field for test conditions (till as applicable)	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test conditions: Two 250-mL glass beakers that contained 200 mL of test solution were used per treatment. The 250-mL test vessels were

	loosely covered to reduce entry of dust, etc. Light: 16-hour light per day using cool-white fluorescent lights with an intensity of 20 uEin/m. Test temperature: 20.3 – 20.7 C Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer and stored in a polyethylene tank where it was aerated. TOC levels were 2 mg/L at the beginning and end of the test and <10 mg/L at the end of the test. Water chemistry: Dissolved oxygen: 7.8 - 8.8 mg/L; pH: 7.4 - 8.5; conductivity: 850 – 900 umhos/cm. Element: Immobilization/mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates: 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test. Test Findings: At 24 hours, no immobilized or dead organisms were observed in the control or at 300 and 1,000 mg/L, but 5% immobilization /mortality was seen in the 100-mg/L treatment. At 48-hours 0, 10, 0, and 0% immobilization were reported for control, 100, 300, and 1,000 mg/L, respectively. Calculation of EL_{50} s: Statistical analysis of survival data not warranted. Exposure period: 48 hours Analytical Monitoring: TOC levels were 2.3 – 2.8 mg/L in the control, 2.8 - 2.9 mg/l at 100, 2.6 - 2.8 mg/L at 300 mg/L, and 2.6 - 3.0 mg/L at 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	Nominal concentrations: 48-h EL_{50} >1,000 mg/L. This is equivalent to 48-h EL_0 = 1000 mg/L based on WAF loading rates.
Remarks	Measured concentration: n/a

	Unit: mg/L Statistical results: Not applicable because immobilization seen at high dose was not significant. Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	The test material was not toxic to daphnids at loading rates tested. Percent survival/unaffected test organisms was 100% in the control, 90% at 100, 100% at 300, and 100% at 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #605 to the Daphnid, *Daphnia magna* T.R. Wilbury Study #9178-CMA/ESI-605.
Other	Updated: 9-6-00

Robust Summary 3-Aquatic Invertebrate Tox: 3

Test Substance	
CAS #	7 1786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts
Remarks	This substance is referred to as alkaryl magnesium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1985, 1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1993
Species/Strain	Cladoceran, *Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (O-h) test solutions and at test termination (4&h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1.
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis of data not warranted because there was no immobilization occurred in this study.
Remarks field for test conditions (fill as applicable)	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test conditions: Two 250-mL glass beakers that contained 200 mL of test solution were used per treatment. The 250-mL test vessels were

	loosely covered to reduce entry of dust. Light: 16-hour light per day using cool-white fluorescent lights with an intensity of 20 uEin/m^2. Test temperature: 19.7 – 20.9 C Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer and stored in a polyethylene tank where it was aerated. TOC levels were 2 mg/L at the beginning and end of the test and <10 mg/L at the end of the test. Water chemistry: Dissolved oxygen: 7.9 - 9.3 mg/L; pH: 7.0 - 8.4; conductivity: 870 – 9 10 umhos/cm. Element: Immobilization/mortality Test Levels: Control, 100, 300, and 1,000 mg/L WAF loading rates: 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test. Calculation of EL_{50} s: Statistical analysis of survival data not warranted. Exposure period: 48 hours Analytical Monitoring: TOC levels were 1.8 – 2.8 mg/L in the control, 2.2 3.3 mg/l at 100, 2.1 - 3.2 mg/L at 300 mg/L, and 1.8 - 3.3 mg/L at 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	Nominal concentrations: 48-h EL_{50} >1,000 mg/L. This is equivalent to 48-h EL_0 = 1000 mg/L based on WAF loading rates (no immobilization noted).
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Not applicable because there was no immobilization.

	Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	The test material was not toxic to daphnids at loading rates tested. Percent survival/unaffected test organisms was 100% in the control, 100,300, and 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #609 to the Daphnid, *Daphnia magna.* T.R. Wilbury Study #9178-CMA/ESI-609.
Other	Updated: 9-6-00

4.3 Toxicity to Aquatic Plants (e.g. Algae)

Robust Summary 3-Aquatic Plant Tox - 1

Test Substance	
CAS #	115733-09-O
Chemical Name	Benzenesulfonic acid, C 14-C24 branched and linear alkyl derivatives, calcium salts
Remarks	This substance is referred to as C14-C24 alkaryl calcium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1985, 1987), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1994
Species/Strain	Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/mL)	approximately 10,000 cells/ml
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements of initial (0-h) high, low and control test solutions and at test termination (96-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 4 15.1
Statistical methods	A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from a log-growth phase in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from University of Texas at Austin alga collection. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of

	water and test substance with the lower end l-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum -10,000 cells/mL. The 250-mL Erlenmeyer flasks were stoppered with foam plugs to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of 47-50 uEin/m^2sec 24-h per day. Test temperature: 23.3 to 24.0 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.O and <10 mg/L, respectively. Test media pH was 7.5 - 9.8 at O-hour and 8.3 - 10.1 after 96 hours. Test Levels: Control, 100, 300 & 1000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Calculation of EL_{50}s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate EC_{50}s (i.e., EL_{50}s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level. Method of calculating mean measured concentrations: not applicable Exposure period: 96 hours Analytical monitoring: At the beginning and end of the test, TOC measurements were between 2 - 8 mg/L in control, 2 – 4 mg/L at 100 mg/L, 2 – 3 mg/L at 300 mg/L and 1,000 mg/L. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.

Results	Nominal concentrations: 72- & 96-h EL_{50} >1,000 mg/L and 72- & 96-h NOEL= 1,000 mg/L based on both growth rate and biomass measurements.
Remarks	Measured concentration: n/a Unit: mg/L Test Findings: At 72-hours biomass measurements in the treatments were 80%, 62%, and 70% of the control at 100, 300, and 1,000 mg/L, respectively. At 96-hours biomass measurements were 70, 66, and 88% of the control at 100, 300 and 1,000 mg/L, respectively. Other: • Test results reported in original study as "effect concentrations" and "no observed effect concentrations" are reported in this summary as "effect loading" and "no observed effect levels", respectively, because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	The test material was not toxic to freshwater alga at loading rates up to and including 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #604 to the Freshwater Alga, *Selenastrum capricornutum*. T.R. Wilbury Study #73-CM-604. Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
Other	Updated: 9-6-00

Robust Summary 3-Aquatic Plant Tox-2

Test Substance	
CAS #	7 1486-79-S
Chemical Name	Benzenesulfonic acid, mono-C15-30-branched alkyl and di-C11-13-branched and linear alkyl derivatives, calcium salts, overbased
Remarks	This substance is referred to as mixed C15-C30 and Cl 1-13 alkaryl calcium salt, overbased derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1985, 1987), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1994
Species/Strain	Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricomutum*
Element basis (# of cells/ml)	approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements of initial (O-h) high, low and control test solutions and at test termination (96-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 4 15.1
Statistical methods	The computer program of Stephan (1983) was used to calculate EL_{50}s. A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect concentration.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from a log-growth phase in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from University of Texas at Austin alga collection. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end l-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.

	Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum -10,000 cells/ml. The 250-mL Erlenmeyer flasks were stoppered with foam plugs to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of 47-50 uEin/m^2sec 24-h per day. Test temperature: 24.0 to 24.2 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test media pH was 7.3 - 10.8 at O-hour and 9.7 - 10.8 after 96 hours. Test Levels: Control, 100, 300, 1000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Calculation of EL_{50} s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate EC_{50}s (i.e., EL_{50}s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level. Method of calculating mean measured concentrations: not applicable Exposure period: 96 hours Analytical monitoring: At the beginning and end of the test, TOC measurements were between non-detect (<1) - 3 mg/L in control, 1 – 2 mg/L at 100 mg/L, 3 mg/L at 300 mg/L, and 5 – 6 mg/L at 1,000 mg/L. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	Nominal concentrations: 72- & 96-h EL_{50} >1,000 mg/L, based on both growth rate and biomass measurements. 72- & 96-h NOEL = 1000 mg/L.
Remarks	

	Measured concentration: n/a Unit: mg/L Test Findings: At 72-hours biomass measurements in the treatments were 133%, 75%, and 64% of the control at 100, 300, and 1,000 mg/L. At 96-hours biomass measurements were 93, 77, and 52% of the control at 100, 100% at 300, and 100% at 1,000 mg/L. Statistical results: A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level. Other: • Test results reported in original study as "effect concentrations" and "no observed effect concentrations" are reported in this summary as "effect loading" and "no observed effect levels", respectively, because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	The test material was not toxic to freshwater alga at loading rates up to and including 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #605 to the Freshwater Alga, *Selenastrum capricornutum*. T.R. Wilbury Study #73-CM-605. Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
Other	Updated: 9-6-00

Robust Summary 3-Aquatic Plant Tox-3

Test Substance	
CAS #	71786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts
Remarks	This substance is referred to as alkaryl magnesium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1985, 1987), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1994
Species/Strain	Freshwater algae, *Pseudokirchneriella suhcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/ml)	approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements of initial (O-h) high, low and control test solutions and at test termination (96-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Statistical methods	The computer program of Stephan (1983) was used to calculate EL_{50}s. A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level.
Remarks field for test conditions (till as applicable)	Test Species: Cells taken from a log-growth phase in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from University of Texas at Austin alga collection. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (I-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end l-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.

	Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum -10,000 cells/ml. The 250-mL Erlenmeyer flasks were stoppered with foam plugs to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of 47-50 uEin/m^2sec 24-h per day. Test temperature: 24.0 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test media pH was 7.5 – 9.9 at O-hour and 8.3 - 10.8 after 96 hours. Test Levels: Control, 125, 250, 500, 1,000 and 1,500 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Calculation of EL_{50} s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate EC_{50}s (i.e., EL_{50}s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level. Method of calculating mean measured concentrations: not applicable Exposure period: 96 hours Analytical monitoring: At the beginning and end of the test, TOC measurements were non-detect (<1) - 1 mg/L in control, 2 – 3 mg/L at 125 mg/L and between 5 – 6 mg/L at 1,000 mg/L. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.

Results	Nominal concentrations: 72-h EL_{50}s = 1,400 mg/L and >1,500 mg/L based on biomass and growth rate, respectively. 96-h EL_{50}s = 1,100 mg/L and > I ,500 mg/L, based on biomass and growth rate, respectively. The 72-h and 96-hr NOEL = 1,000 mg/L.
Remarks	Measured concentration: n/a Unit: mg/L Test Findings: At 72-hours biomass measurements in the treatments were 97, 100, 101, 95, and 35% of the control at 125, 250, 500, 1,000, and 1,500 mg/L, respectively. At 96-hours biomass measurements were 78, 61, 62, 58, and 31% of the control at 125, 250, 500, 1,000. and 1,500 mg/L, respectively. Logarithmic growth was observed at all treatments up to and including 1,000 mg/L; i.e., average biomass measurements 1,383 – 1,860 cells/mL $x10^3$. Therefore, the 96-h NOELs were determined to be 1,000 mg/L although statistical analysis determined the 96-h NOELs to be 125 mg/L. But, the hypothesis test was biased towards the unusually high control growth (2,383 cells/mL $x 10^3$) between 72 96 hours upon which test concentration growth was compared. This produced an erroneous measurement of NOEL. Other: • Test results reported in original study as "effect concentrations" and "no observed effect concentrations" are reported in this summary as "effect loading" and "no observed effect levels", respectively, because test results are based on WAF loading rates. • Effects were determined to be algistatic based on the rapid re-growth of an aliquot of cells taken from 1,500 mg/L cultured in fresh control media. • Control response was satisfactory.
Conclusions	The test material was not toxic to freshwater alga at loading rates up to and including 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #609 to the Freshwater Alga, *Selenastrum capricornutum*. T.R. Wilbury Study #73-CM-609. Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
Other	Undated: 9-6-00

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Robust Summary 3-Acute)ral -1

Test Substance	
CAS #	CAS# Analog of 70024-69-O
Chemical Name	C20-24 alkaryl calcium salt derivative
Remarks	Test material-dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 40 1
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1989
Species/Strain	Rats/Sprague-Dawley strain
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5 g/kg
Dose volume	Not provided
Control group included	Yes
Remarks field for test conditions	A single dose of 5.0 g/kg of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats. An untreated control group of S/sex was included. The animals were observed for signs of physiological or behavioral changes frequently on the day of treatment. Thereafter all animals were examined for signs of toxicity once per day. Individual body weights were recorded on the day of dosing and at 2, 7 and 14 days after dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Diarrhea and reduced food intake were observed in one treated female on Day 1. No other signs of toxicity were observed. Body weights were unremarkable.

Conclusions	The test article, when administered as received to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 5.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/10/00 (RTA-004)

Robust Summary 3-Acute Oral-2

Test Substance	
CAS #	CAS# 61789-86-4
Chemical Name	Petroleum derived calcium salt
Remarks	100% purity
Method	
Method/Guideline followed	OECD Guideline 40 1
Test Type	Acute oral toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Rats/Sprague-Dawley, Crl:CD® (SD)BR
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	Peanut oil
Route of administration	Oral (intragastric)
Dose level	5 g/kg
Dose volume	7 ml/kg
Vehicle control group	Yes
Chemical analysis of dosing solution	**Yes**
Remarks field for test conditions	A single dose of 5.0 g/kg of the test material diluted in peanut oil at a concentration of 7 14 mg/ml was administered intragastrically to five fasted (over night) male and female rats. The concentration of the test material in the vehicle was analyzed for homogeneity and for stability. The test material was administered at a dose volume of approximately 7 ml/kg body weight. A vehicle control group consisting of 5-fasted animals/sex was dosed with 7 ml/kg of peanut oil. The animals were observed for signs of toxicity or behavioral changes frequently on the day of treatment. Thereafter, all animals were examined for signs of toxicity twice per day (once/day on weekends), for the 13-day observation period and once on day 14 prior to sacrifice. Individual weights were recorded immediately prior to dosing and at 2, 7 and 14 days after dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days. Histopathological evaluations were nerformed on grossly abnormal tissues only.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	Analysis confirmed that the dosing solution was homogeneous and stable for the period of use and that it was prepared at the appropriate concentration. No deaths were observed during the 14-day observation period. Diarrhea was observed in one treated male and in one control male 5 hours post dosing, only. Alopecia with or without

	thinned fur was seen in one vehicle control male (Days 12-14) and in one vehicle control female (Days 10- 13) Other than the previous observations, all animals appeared normal throughout the 14-day observation period. No body weight effects occurred. No test material related macroscopic or microscopic findings were evident.
Conclusions	The test article, when administered in peanut oil to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 5.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Last-dated:. 2/9/00 (RTA-001)

Robust Summary 3-Acute Oral-3

Test Substance	
CAS #	CAS# 6 1790-48-5
Chemical Name	Petroleum derived barium salt
Remarks	Test material dosed as received, purity is 46 % active.
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1982
Species/Strain	Rats/Sprague-Dawley strain
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	2 g/kg
Dose volume	***2.4*** ml/kg
Control group included	No
Remarks field for test conditions	A single dose of 2.0 g/kg of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats. A control group was not included. The animals were observed for signs of toxicity or behavioral changes frequently on the day of treatment. Thereafter, all animals were examined for signs of toxicity twice per day. Individual weights were recorded on the day of dosing and weekly thereafter. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 > 2.0 g/kg (males and females)
Remarks	One treated female died on Test Day 5 without exhibiting any clinical symptoms. All remaining animals survived to study termination. The animals exhibited ruffled fur 3 hours post dosing. Urine staining was observed within 24-48 hours of dosing. After 72 hours all animals essentially recovered. No body weight effects were observed. Gross necropsy findings were unremarkable for all animals.
Conclusions	The test article, when administered as received to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 2.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
Re ***erences***	Unpublished confidential business information
Other	Updated: 2/10/00 (RTA-003)

Robust Summary 3-Acute Oral-4

Test Substance	
CAS #	CAS# 68608-26-4
Chemical Name	Petroleum derived sodium salt
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	**Y**
Year (Study Performed)	1983
Species/Strain	Rats/Sprague-Dawley strain
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5 g/kg
Dose volume	5 ml/kg
Control group included	**No**
Remarks field for test conditions	A single dose of 5.0 g/kg of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats. A control group was not included. The animals were observed for signs of toxicity or behavioral changes frequently on the day of treatment. Thereafter, all animals were examined for signs of toxicity twice per day. Body weights were recorded on the day of dosing and weekly thereafter. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 davs.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No deaths or clinical signs of toxicity were observed during the 14-day observation period. Thetreated males exhibited a slight body weight decrease during the first week post dosing. These body weights recovered during the second week. Body weight gain in the females was normal at week 1 but was less than expected during the second week of the study. Gross necropsy findings were unremarkable.
Conclusions	The test article, when administered as received to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 5.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/16/00 (RTA-036)

Robust Summary 3-Acute Oral-5

Test Substance	
CAS #	CAS# 68783-96-O
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 40 1
Test Type	Acute oral toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1984
Species/Strain	Rats/Sprague-Dawley
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5 g/kg
Dose volume	4.3 ml/kg
Control group included	Yes
Remarks field for test conditions	A single dose of 5.0 g/kg of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats. Five fasted undosed animals of each sex served as the controls. The animals were observed frequently for any physiological or behavioral abnormalities on the day of dosing and at least twice each weekday for 13 days after treatment. On weekends, observations were made once daily. On day 14 the animals were observed once prior to sacrifice. Individual body weights were recorded on the day of dosing and on days 2, 7 and 14 after dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals on Day 14.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Body weights were unremarkable. Slightly reduced food consumption was observed in one treated male (Day 2) and female (Day 1). There were no macroscopic findings associated with treatment.
Conclusions	The test article, when administered as received to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 5.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/17/00 (RTA-018)

Robust Summary 3-Acute Oral-6

Test Substance	
CAS #	CAS# 7 1549-79-6
Chemical Name	Mixed C 15-C30 and C 1 1- 13 alkaryl derivative
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1978
Species/Strain	Rats/Sherman-Wistar strain
Sex	Male
No. of animals/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	1, 2, 4, 8, and 16 ml/kg
Dose volume	1, 2, 4, 8, and 16 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted (over night) male rats at each treatment level. A control group was not included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 14.9 g/kg (males)
Remarks	Three 16.0 g/kg animals died on test day 3. Animals at this dose level were depressed at l-hour post dosing and remained in poor health for approximately 7 days before recovering. A reduced mean body weight compared to the other treated groups was observed in this group at termination. No clinical findings or body weight effects were evident in the other dose groups. Gross necropsy findings were unremarkable for all animals.
Conclusions	The test article, when administered as received to male Sherman-Wistar rats, had an acute oral LD50 of 14.9 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/1 5/00 (RTA-035)

Robust Summarv 3-Acute Oral-7

Test Substance	
CAS #	CAS# 7 1786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	FHSA 16CFR 1500.3
t Test Type x i c	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1980
Species/Strain	Rats/Sherman-Wistar strain
Sex	Male
No. of animals /dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	1 .0, 2.0, 4.0, 8.0 and 16 g/kg
Dose volume	1.0, 2.0, 4.0, 8.0 and 16 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted (over night) male rats at each dose level. A control group was not included. All animals were examined for signs of toxicity daily for 14 days. Individual weights were recorded on the day of dosing and at termination. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 > 16.0 g/kg (males)
Remarks	No deaths were observed during the 14-day observation period. The animals at 8 and 16 g/kg exhibited ruffled fur for 18-24 hours post dosing. Within 48 hours all animals appeared normal. No body weight effects were observed. Gross necropsy findings were unremarkable.
Conclusions	The test article, when administered as received to male Sherman-Wistar rats, had an acute oral LD50 of greater than 16.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/16/00 (RTA-034)

5.1.2 Acute Inhalation Toxicity

Robust Summary 3-Acute nhalation-1

Test Substance	
CAS #	CAS# 68783-96-O
Chemical Name	Petroleum derived calcium salt, overbased
Purity	35.1 % active in oil
Method	
Method/Guideline followed	OECD Guideline 403
Test Type	Acute Inhalation toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Rats/Sprague-Dawley
Sex	Male and female
No. of animals/sex	5
Vehicle	Oil based material dosed undiluted
Route of administration	Aerosol inhalation (single 4 hour whole body exposure)
Dose level	1.9 mg/L (actual maximum attainable concentration)
Vehicle control group	No
Chamber analysis	**Yes**
Remarks field for test conditions	One group of five rats/sex was exposed for 4 hours to the test material as a liquid droplet aerosol generated by a pressure spray apparatus delivered into a 1 OO-liter plexi-glass exposure chamber. The actual exposure concentration as measured by gravimetric analysis was 1.9 mg/L. Particle size analyses were performed once/hour using a multi-stage cascade impactor. Animal observations for toxicological signs and mortality were recorded periodically during exposure and twice daily during the 14 day observation period. Individual body weights were recorded on Day1 (immediately prior to exposure) and on Days 2, 3, 5, 8 and 15. Animals were euthanized by exsanguination under ether anesthesia. All animals were subjected to a complete gross necropsy.
Results	LC50 > 1.9 mg/L (males and females)(maximum attainable concentration)
Remarks	The mass median aerodynamic diameter was 4.2 microns with a geometric standard deviation of 1.9 (estimated percent of particles < 10 microns=93%). All animals survived the exposure and observation periods. Observations recorded during exposure included reduced activity, matted coat and closed eyes. Observations noted post exposure on Day 1 included lacrimation, nasal discharge, salivation, , rales, matted coat, hunched appearance, soft stool and closed eyes. These findings decreased in incidence over the next week. Animals

	were free of symptoms of exposure during the second week of observation. Several animals exhibited very slight body weight losses on Day 2. Body weights recovered and were unremarkable by Day 5. There were no abnormal postmortem findings evident in any of the animals at study termination.
Conclusions	Following 4-hour whole body exposure to a liquid droplet aerosol of the test material the LC50 in male and female Sprague Dawley rats was >1.9 mg/L. This was the maximum concentration attainable.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/18/00 (RTA-023)

5.1.3 Acute Dermal Toxicity

Robust Summary # 3-Acute Dermal-1

Test Substance	
CAS #	CAS# 115733-09-o
Chemical Name	C 14-24 alkarvl calcium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1981
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	5 g/kg
Dose volume	5 ml/kg
Control group included	**No**
Remarks field for test conditions	This study was conducted prior to the development of Test Guideline 402. This study deviated from Guideline 402 in that animals were abraded prior to dosing. This deviation was not considered sufficient to change the outcome of the study. Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. On the day of dosing the skin was abraded prior to test material administration. A single dose of 5 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze patch and elastic bandage. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs frequently on the day of dosing and once daily for 14 days after treatment. Individual body weights were recorded on the day of dosing and on days 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.
Results	LD50 > 5.0 g/kg (males and females
Remarks	Rabbits were normal at 0, 2 and 4 hours post dosing. Upon bandage removal at 24 hours rabbits were distressed. Skin at the dose site was red, swollen and stained with test material. Irritation subsided by day 9, however the skin remained dry, flaky and stained throughout the observation period. All animals

	gained weight during the study. No systemic toxicity was observed. At necropsy 9 rabbits exhibited alopecia, matted fur and flaky skin at or around the test site. One animal had a friable, white, mottled left front liver lobe. One rabbit had a small right testis.
Conclusions	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 5.0 g/kg. No evidence of systemic toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/16/00 (RTA-033)

Robust Summary 3-Acute Dermal-2

Test Substance	
CAS #	CAS# 6 1789-86-4
Chemical Name	Petroleum derived calcium salt
Remarks	Test material purity 100%
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	5 g/kg
Dose volume	5 ml/kg
Control group included	Yes
Remarks field for test conditions	Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. Elizabethan type collars were placed on the neck of each rabbit. The skin was left intact. Collars remained on for 24 hours post dosing. Animals were reclipped as needed. A single dose of 5 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a plastic wrap, which was over wrapped with paper toweling. The application site was wiped cleaned of residual test material at the end of the 24-hour exposure period. Five clipped, untreated animals/sex were wrapped as described above and served as sham controls. The animals were observed for abnormal clinical signs frequently on the day of dosing and twice daily for 13 days after treatment. On Day 14 the animals were observed once prior to sacrifice. Dermal examinations (Draize) were performed on day 1, 7 and 14. Individual body weights were recorded on the day of dosing and on days 2, 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14. Selected tissues, including the skin were examined microscopically.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Reduced food intake and nasal discharge were observed in treated and control animals. Slight to moderate erythema and edema were observed in both sexes 24 hours after treatment. Slight erythema was observed in three animals on Day 7. Dry and flaky skin was observed in all treated animals on Days 6 and 14. Body weights were unremarkable. Gross pathological findings included dry, flaky skin at the dose site of all treated

	animals. Microscopic examination revealed the presence of trace to mild hyperkeratosis. There were no other treatment related gross or microscopic findings.
Conclusions	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 5.0 g/kg. No evidence of systemic toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/17/00 (RTA-020)

Robust Summary 3-Acute Dermal3

Test Substance	
CAS #	CAS# 68783-96-O
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1993
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	,
Dose volume	1.8 ml/kg
Control group included	No
Remarks field for test conditions	Approximately 24 hours prior to topical application of the test material, the hair of each animal on the dorsal surface from the shoulder region to the lumbar region was closely clipped. Elizabethan type collars were placed on the neck of each rabbit. The skin was left intact. Collars remained on for the duration of the study. Animals were reclipped as needed. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours, on approximately 10% of the total body surface under a semi-occlusive bandage that was covered with an elastic bandage. The application site was wiped clean of residual test material with water at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs 2 and 4 hours after dosing and once daily for the 14-day study period. Cutaneous examinations (Draize) were performed on day 1 (45 minutes after patch removal) and on Days 3, 7, 10 and 14. Individual body weights were recorded on the day of dosing and on day 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.
Results	LD50 > 2.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical observations were unremarkable in 7 of 10 treated animals. Three treated animals exhibited findings consistent with stress and collaring. Findings included sores/scabs in the mouth and in the dorsal cervical area and stool abnormalities. Erythemia was observed in all animals on day 1. Very slight to well defined erythemia was observed in 9 of 10 treated animals on Day 3. On Day 7 one animal exhibited slight erythemia and one

	animal exhibited well-defined erythemia. Slight erythemia was observed in two animals on Days 10 and 14. Edema was not observed in any of the animals. Desquamation was observed in all animals on Day 7 By Day 14 desquamation was observed in six treated animals. All animals exhibited body weight gains during the treatment period. At necropsy 6 of 10 treated animals exhibited desquamation. One animal was noted with tan striations on the liver.
Conclusions	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 2.0 g/kg. No evidence of systemic toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2117100 (RTA-010)

Robust Summary 3-Acute Dermal-4

Test Substance	
CAS #	Analog of CAS# 70024-69-o
Chemical Name	C20-C24 alkaryl calcium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1989
Species/Strain	Rats/Sprague Dawley
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg
Dose volume	Not specified
Control group included	Yes
Remarks field for test conditions	Approximately 24 hours prior to topical application of the test material, the hair of each control and treated animal was closely clipped. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a plastic film and elastic bandage. Plastic collars were used during the dosing phase. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs frequently on the day of dosing and once daily for 14 days after treatment. Individual body weights were recorded on the day of dosing and on days 2, 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.
Results	LD50 > 2.0 g/kg (males and females)
Remarks	No signs of systemic toxicity were observed. All treated animals exhibited skin irritation. Significant differences in mean body weight were observed between treated and control males on Days 2, 7 and 14. At necropsy, multiple pinpoint scabs were observed in three treated males and one treated female.
Conclusions	The test article, when administered dermally as received to 5 male and 5 female Sprague Dawley rats had an acute dermal LD50 of greater than 2.0 g/kg. No evidence of systemic toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 10/4/00 (RTA-068)

5.2 Repeated Dose Toxicity

Robust Summary 3-Four Week Oral 1

Test Substance	
CAS #	Analog of CAS# 70024-69-o
Chemical Name	C20-24 alkaryl calcium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 407
Test Type	28-day oral toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1989
Species	Rat
Strain	Sprague-Dawley CD, 4 1 days old at initiation of treatment
Route of administration	Oral gavage (syringe and dosing tube)
Duration of test	29 days of treatment followed by 14 day recovery period in the control and high dose satellite recovery groups
Doses/concentration levels	0, 100, 500 and 1000 mg/kg/day
Sex	Males and females
Exposure period	29-day treatment duration with a 14 day recovery
Frequency of treatment	7 days/week
Control group and treatment	6 rats/sex/group for each dose, and satellite recovery groups of 6 animals/sex for the control and 1000 mg/kg/day dose. Control group received daily doses of peanut oil at 2.0 ml/kg, and treatment groups received the indicated dose of test material diluted in peanut oil at a dose volume of 2.0 ml/kg
Post exposure observation period	14-days
Statistical methods	Body weight, food consumption, feed efficiency, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's post-hoc test, non-parametric Kruskal-Wallis and a Mann-Whitney U-test, Bartlett's test for equal variances, a Student's t-test and Dixon's test for rejection of outlying values.
Dose rangefinding study	Yes (Pilot two-week repeated dose oral toxicity study)
Remarks field for test conditions	Single oral doses were administered for 29 consecutive days using a gavage needle. Clinical observations were made daily. Viability checks were performed twice daily. Body weight were recorded twice weekly during treatment and weekly during recovery. Terminal body weights were recorded. Food consumption were recorded during treatment and recovery. Hematology, clinical chemistry and urinalysis parameters were evaluated at termination of

	treatment and recovery. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically. Significant deviations from the OECD 407 test guidelines include: • A function observational battery for neurotoxicity was not performed since this test was not part of the OECD 407 guideline at the time the study was performed. • Microscopic pathology was performed as required by OECD 407 guideline in place at the time the study was conducted.
Results	
Remarks	An NOEL of 500 mg/kg/day was established for this study. No test material related mortality was observed. One low dose male was found dead on Day 9. This was attributed to a probable misdosing. A second low dose male was replaced, due to a possible misdosing, on the first day of treatment. Mean serum cholesterol levels were significantly reduced in the 1000 mg/kg males and females at termination of dosing and in the 1000 mg/kg females at the end of the 14-day recovery period. No treatment-related effects were observed on mortality, clinical observations, body weight and body weight gain, food consumption, feed efficiency, hematology, urinalysis, absolute and relative organ weights and macroscopic or microscopic pathology. Statistically significant differences from control were observed for some hematology and clinical chemistry parameters. These values were within clinically normal limits and were not associated with corresponding histopathological changes. They were not considered biologically significant. Chemical analysis of dosing solutions confirmed that they were homogeneously prepared at the desired concentrations.
Conclusions	Little subchronic toxicity was observed over the range of doses administered in this study. Based on a reduction in mean cholesterol values in the males and females treated at the 1000 mg/kg dose level, the NOEL was 500 mg/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/11/00 (RTA-006)

Robust Summary 3-Four Week Dermal -2

Test Substance	
CAS #	CAS# 68783-96-O
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1995
Species	Rat
Strain	Sprague-Dawley CD, 8-9 weeks of age at initiation of treatment
Route of administration	Dermal, 6 hour/day, to the clipped, unabraided, dorsal surface.
Duration of test	28 days of treatment followed by 14 day recovery period in the high dose satellite recovery group only.
Doses/concentration levels	0, 100, 300 and 1000 mg/kg/day
Sex	Males and females
Frequency of treatment	Once/day, 7 days/week
Control and treatment groups	5 rats/sex in the control group, in each dose level and in the satellite recovery group at the 1000 mg/kg/day dose. The control group received no treatment (sham control). The test material was administered undiluted to the treated animal based on individual animal body weight.
Post exposure observation period	14-days (High dose group only)
Dose rangefinding study	Dose levels were selected based on results of a rangefinding study conducted at dose levels up to 1 000mg/kg/day. No signs of toxicity were observed.
Statistical methods	Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend. A Student's I-test was used to compare the satellite group's main study termination and recovery blood values and organ weights.
Remarks field for test conditions	The test material was applied to the clipped, unabraided dorsal surface of the rats for 6 hours/day, 7 days/week for 28 days. The gauze patch was secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. After at least 6 hours the test material residue was removed from the skin with peanut oil and a paper towel. Clinical observations were made daily. Dermal responses were evaluated (Draize) prior to dosing on days 0, I, 4, 7, 11, 14, 18, 2 l, and 25; prior to blood collection on day 28 and after sleeve removal on day 0. Satellite animals were also evaluated on Days 32, 35, 40 and 42. Body weight and food consumption were recorded during treatment and recovery.

	Hematology and clinical chemistry parameters were evaluated at termination of treatment and recovery. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically.
Results	
Remarks	A NOAEL of 1000 mg/kg was established for this study. No mortality occurred during this study. Low incidences of very slight erythemia, desquamation and/or pinpoint scabbing were observed sporadically in the treated animals. All animals were free of edema during the study. Body weights and food consumption data were unremarkable during the treatment and recovery periods. There were no treatment-related differences from control observed in the hematology data of the treated animals following the dosing or recovery periods. Differences from control were noted for several hematology parameters including a statistically significant increase in the mean percentage of neutrophils of the 300 and 1000 mg/kg females and a decrease in mean percentage of lymphocytes in the 1000 mg/kg females compared to control on Day 28. There was a statistically significant decrease in mean percentage of basophils in the satellite females from Day 28 to 42. However these values were within the normal range. In the absence of differences from control in absolute white blood cell counts, these findings were considered unrelated to treatment. There was a statistically significant decrease in the mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration of the male satellite animals from Day 28 to 42. In the absence of other significant findings in mean hemoglobin or red blood cell parameters, these small differences were not considered clinically significant. Serum chemistry values were unremarkable in the treated animals at termination of the treatment and recovery periods. There was a slight increase in the mean aspartate aminotransferase and alanine aminotransferase of the high dose females at Day 28. These increases were attributed to two females with high values. Similar changes were not observed in the satellite females or in the males at Day 28. These increases were not considered related to treatment. There were several differences from control noted at the end of recovery. These values were within the range of normal and similar differences were not evident at the end of the treatment period indicating that these findings were not clinically significant or treatment related. Gross postmortem findings were limited to one 300 mg/kg male with small testes, one control female with discolored lungs and liver and black material in the stomach; and single occurrences of scabs in the 100 and 1000 mg/kg and recovery males. These findings were considered incidental and unrelated to treatment. Tape irritation was observed in a number of animals. There were no alterations in organ weights that were attributed to treatment with the test material. Slight alterations were noted in several organ weights at termination of dosing or recovery. There was a statistically significant decrease in mean absolute brain weight of the 300 mg/kg females compared to control. This finding lacked a dose response and was not considered biologically significant. There was a statistically significant decrease in mean relative adrenal and testes weights of the male satellite animals at termination of recovery compared to control at end of treatment. Compared to the high dose at study termination there was a statistically significant decrease in mean relative

	adrenal, brain and testes weight of the male satellite animals and mean relative adrenal and brain weight of the female satellite animals at recovery termination. These alterations in organ weights were attributed to the cessation of the stress associated with wrapping (adrenal) and the animals continued increase in body weight while organ weights remained constant in adult animals. In the absences of significant organ weight findings following treatment or correlating effects with histopathology these findings were not considered clinically significant. There were no test material related microscopic findings noted in any group. Livers from female rats of all groups (including control) sacrificed after 28 days of treatment exhibited focal necrosis. This finding did not exhibit a dose response. This finding has been seen in other dermal studies and has been attributed to trauma and/or ischemia to the liver resulting from the wrapping and manipulation of the animals. Liver necrosis was not evident in any of the satellite recovery animals. This finding was not considered treatment related. The treated skin of most animals revealed variable amounts of thickening of the epidermis due to acanthosis and hyperkeratosis, sebaceous gland hyperplasia and focal dermal inflammation. These changes occurred in all groups including control. However the severity of these changes tended to be increased in the male treated group rats and in the females of the 300 and 1000 mg/kg groups, suggesting a mild irritating effect of the test material. Following recovery these findings were less severe.
Conclusions	A NOAEL of 1000 mg/kg was established for this study. Under the conditions of this study dermal application of this test material resulted in no signs of overt systemic toxicity.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/18/00 (RTA-024)

Robust Summary 3-Four Week Inhalation-3

Test Substance	
CAS #	CAS# 68783-96-O
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	35.1% active in oil
Method	
Method/Guideline followed	OECD 412
Test Type	28-day inhalation toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1986
Species	Rat
Strain	Sprague-Dawley CD, 6-7 weeks of age at initiation of treatment
Route of administration	Aerosol inhalation, whole body exposure
Duration of exposure	6 hours/day
Doses/concentration levels	49.5, 156, 260 mg/m³ (measured concentration)
Sex	Males and females
Frequency of treatment	5 days/week for 4 weeks
Control and treatment groups	5 rats/sex
Post exposure recovery period	None
Statistical methods	Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend
Dose rangefinding study	No
Remarks field for test conditions	Treated animals were exposed to the test material as a liquid droplet aerosol generated by a pressure spray apparatus delivered into a 1000-liter glass and stainless steel exposure chamber. Chamber airflow rates was approximately 200 liters/minute with a chamber 99% equilibration time of 22 minutes. Control animals were exposed to room air only. Chamber exposure concentrations were measured by gravimetric analysis at one and one half-hour intervals. Particle size analyses were performed once/week using a multi-stage cascade impactor. Animal observations for toxicological signs and mortality were recorded periodically during exposure and twice daily during the 14 day observation period. Individual body weights were recorded weekly. Hematology and clinical chemistry evaluations were performed on all animals prior to terminal sacrifice. Animals were euthanized by exsanguination under ether anesthesia. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically.

Results	
Remarks	The actual exposure concentrations measured by gravimetric analysis were 49.5, 156, 260 mg/m^3. The mass median aerodynamic diameter of the aerosol ranged from 3.3 to 3.7 microns, with an average geometric standard deviation range of 2.0 to 2.1. These data confirmed that the aerosol was respirable in the rat (estimated percent of particles <10 microns=93%). There was no test material exposure related mortality during the study. One low dose animal escaped from its cage and was euthanized. One control male died during blood collection immediately prior to its scheduled sacrifice. Red nasal discharge, matted coat and decreased activity were noted at the two higher concentrations. The mean body weight gain of the high dose males was slightly reduced over the four weeks of study. Body weights and gains in the other groups were unremarkable. Clinical chemistry and hematology data exhibited no patterns indicative of a treatment-related effect. Several incidental statistically significant differences from control were observed these included: increased hematocrit (low dose females), creatinine phosphokinase (low and high dose females) and sodium (high dose females). These differences were not attributed to treatment. Dose related increases in absolute and relative (to body weight) lung weights were observed in the mid and high dose males and females. Increases were statistically significant, with the exception of mid dose female absolute lung weight. Microscopically the accumulation of intraalveolar macrophages (males: 3,5,5,5; females: 5,5,5,5) and hyperplasia/hypertrophy of bronchiole epithelium (males: 3,4,5,5; females: 4,5,5,5) were seen in the control and treated groups. While these findings were observed in control and treated animals the severity of the lesions exhibited a dose response in the mid and high dose groups and was considered treatment related. Differences in severity between the control and low dose group were equivocal. Based on these findings the lowest dose level (49.5 mg/m^3) is considered the NOAEL by this reviewer.
Conclusions	Under the conditions of this study inhalation exposure of this test material resulted in minimal toxicity over the range of doses administered. A NOAEL of 49.5 mg/m^3 was established for this study based on the slight, dose related increase observed in the severity of microscopic pulmonary findings and increased lung weights.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/24/00 (RTA-025)

Robust Summary 3-Four Week Derma14

Test Substance	
CAS #	CAS# 7 1786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rabbits
GLP (Y/N)	Y
Year (Study Performed)	*1981*
Species	Rabbit
Strain	New Zealand White (SPF) (approximately 2 kg in body weight at initiation)
Route of administration	Dermal, 6 hourlday, 5 days/week, to the clipped, unabraided, dorsal surface.
Duration of test	20 days of treatment followed by 4 week recovery period
Doses/concentration levels	0, 25 and 100% (w/v) (OECD Guideline 4 10 suggests three treated groups and a control be included in this study design. The lowest dose level should be free of toxic effects. These suggestions were not met in this study.)
Vehicle control	Prim01205
Dose volume	2 ml/kg/day
Sex	Males and females
Frequency of treatment	Once/day, 5 days/week for a total of 20 doses.
Vehicle control and treatment groups	15 rabbits/sex in the vehicle control group and in both treated groups. Five of the initial 15 animals/sex/group served as recovery animals. The control group received the vehicle. An untreated control group was not included in the study. The test material was administered undiluted to the treated animal in the high dose group. The animals in the low dose group received the test material diluted in the vehicle. Doses were administered based on individual animal body weights.
Post exposure observation period	*4* weeks
Statistical methods	Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a **Dunnett's test** and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend.
Remarks field for test conditions	The test material was applied to the clipped, unabraided dorsal surface of the rabbits for 6 hours/day, 5 days/week for 20 days. Elizabethan collars were used to prevent ingestion. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not **used** during this study. This is considered a minor deviation from the Guideline.) After approximately 6 hours the test material residue was removed from the skin with a paper towel, if

	necessary. Clinical observations were made weekly. Dermal responses were evaluated daily during treatment (7 days/week; prior to dosing on dosing days) and recovery. Body weight was recorded weekly during treatment and recovery. (OECD Guideline 410 suggests the recording of food consumption. This parameter was not recorded during this study. This is considered a minor deviation from the guideline.) Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment and recovery. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically in the control and high dose animals sacrificed at the end of the treatment period and for all found dead and moribund sacrifice animals. In addition the liver, testes and epididymides were evaluated in all low dose animals.
Results	
Remarks	One control and four high dose animals died or were sacrificed early during this study. One control female was sacrificed moribund during recovery (test day 35). Two high dose males were sacrificed moribund during the treatment period (test days 23 and 32). One high dose male was found dead (test day 49) during recovery. One high dose female was sacrificed moribund during recovery (test day 39). The **cause** of death of these animals was not established. Alopecia was observed in many of the low and high dose males and females during the last two to three weeks of treatment and during the first two to three weeks of recovery. Several high dose males and females exhibited this finding throughout recovery. Erythema, edema, atonia, desquamation, fissuring and exfoliation were observed in all of the low and high dose animals throughout the treatment period. Most of these findings were evident during recovery with a decreasing severity and incidence. These data did not exhibit a strong dose response. Erythema and desquamation were observed in the control males and females during the treatment and recovery periods. These findings were less severe then those observed in the treated animals. As in the treated groups severity and incidences decreased with time during recovery. The mean body weights of the low dose males and females were slightly lower (-5%) than control during the last two weeks of treatment and during the first week of recovery. The mean body weights of the high dose males and females were lower than control (5- 15%) during the last two weeks of treatment and throughout recovery. Some of the difference from control observed during recovery may be due to the small number of animals (2-5) available in each group and the normal variability expected in rabbit weight. The mean total leukocyte count of the low and high dose males and females were statistically significantly lower than control at termination of the treatment period. Low and high dose males and high dose females were also slightly reduced at the end of recovery. In addition the mean hemoglobin and hematocrit values and the mean erythrocyte **count** of the high dose females were significantly reduced following treatment but not following recovery. The low and high dose males and females exhibited slight or statistically significant, dose-related decreases in total protein and globulin and increased albumin/globulin ratios at termination of treatment. In addition albumin was

	slightly reduced in the high dose females. At termination of the recovery period the mean globulin level of the low dose females was significantly reduced and the albumin/globulin ratios of the low and high dose females were slightly (statistically significantly) increased compared to control. At termination of treatment the low and high dose males exhibited increases in mean SGOT and alkaline phosphatase. The low and high dose females exhibited increases in SGOT and SGPT. These enzymes were unremarkable following recovery. (The changes observed in SGOT and SGPT were not discussed in the original final report of this study.) Treatment related decreases were observed in the absolute and relative (to body weight) testes and epididymides weights of the low and high dose males at the end of the treatment and recovery periods. Absolute testes weights were decreased -21 and -35%, compared to control, in the low and high dose groups following treatment and -22 (low dose) and -58% (high dose) following recovery. Treatment related increases were observed in the absolute and relative (to body weight) liver weights of the low and high dose males (+5/+30%-absolute weight) and females (+ 12/+23%-absolute weight) following treatment and in the high dose males (+14%-absolute weight) following recovery. Macroscopic examinations revealed dermal findings consistent with those observed during the in life examinations. The testes of many low and high dose animals were noted to be small in size at the end of the treatment period. This observation was recorded in one high dose recovery animal. These data are consistent with the reduced testes weights observed in the low and high dose groups. Microscopic evaluations revealed treatment related morphologic changes in the skin, testes, epididymides and possibly the liver. Compound related microscopic lesions were seen in the treated skin of the high dose animals at termination of the treatment period (high dose recovery and low dose treatment and recovery animals were not examined). Treated skin findings included slight to moderately severe hyperkeratosis and epithelial hyperplasia. Findings in males and females were comparable. Possible treatment related liver findings were observed in the high dose group only. Findings present at termination of dosing but not following recovery included the presence of multifocal areas of minimal to moderate hepatocellular degeneration usually accompanied by multifocal areas of necrosis and/or multifocal areas of coarse cytoplasmic vacuolation of hepatocytes. Testicular changes were observed in the high dose males only following treatment and recovery. No changes were evident in the low dose males. Alterations observed in the high dose included aspermatogenesis, reduced numbers of spermatids, and multifocal to diffuse tubular hypoplasia. Epithelial hypoplasia of the epididymis accompanied the testicular changes in many animals at termination of treatment but not following recovery. There were no other findings observed in this study that were considered treatment related.

Conclusions	Based on the findings observed during this study this reviewer has concluded that an NOAEL was not established for this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 4/13/00 (RTA-029)

Robust Summary 3-Four Week Dermal 5

Test Substance	
CAS #	CAS# 7 1786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1995
Species	Rat
Strain	Sprague-Dawley CD, 8-9 weeks of age at initiation of treatment
Route of administration	Dermal, 6 hour/day, to the clipped, unabraided, dorsal surface.
Duration of test	28 days of treatment followed by 14 day recovery period in the high dose satellite recovery group only.
Doses/concentration levels	0, 100, 300 and 1000 m&/kg/day
Sex	Males and females
Frequency of treatment	Onceiday, 7 days/week
Control and treatment groups	5 rats/sex in the control group, in each dose level and in the satellite recovery group at the1000 mg/kg/day dose. The control group received no treatment (sham control). The test material was administered undiluted to the treated animal based on individual animal body weight.
Post exposure observation period	14-days (High dose group only)
Statistical methods	Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend. A Student's t-test was used to compare the satellite groups main study termination and recovery blood values and organ weights.
Dose rangetinding study	Dose levels were selected based on results of a rangefinding study conducted at dose levels up to 1000mg/kg/day. No signs of toxicity were observed.
Remarks field for test conditions	The test material was applied to the clipped, unabraided dorsal surface of the rats for 6 hours/day, 7 dayslweek for 28 days. The gauze patch was secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. After at least 6 hours the test material residue was removed from the skin with peanut oil and a paper towel. Clinical observations were made daily. Dermal responses were evaluated (Draize) prior to dosing on days 0, 1, 4, 7, 11, 14, 18, 21, and 25; prior to blood collection on day 28 and after sleeve removal on day 0. Satellite animals were also evaluated on Days 32, 35, 40 and 42. Body weight and food consumption were recorded during treatment and recovery. Hematology and clinical chemistry parameters were evaluated at termination of

	treatment and recovery. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically.
Results	
Remarks	A NOAEL of 1000 mg/kg was established for this study. No treatment related mortality was observed. One 300 mg/kg female was found dead on Day 19. This death was attributed to the wrapping procedure. One 1000 mg/kg male died following blood collection at study termination. Desquamation was observed in one 300 mgikg female on days 4 and 7. No other significant clinical in life or dermal observations were observed. Body weights and food consumption data were unremarkable during the treatment and recovery periods. There were no treatment-related differences from control observed in the hematology data of the treated animals following the dosing or recovery periods. Differences from control were noted for several hematology parameters including a decrease in mean percentage of eosinophils in low and mid dose males at termination of treatment. These values were within the normal range and did not exhibit a dose response. Following the recovery period there were several statistically significant differences from control noted in the hematology parameters of the satellite animals. These included decreases in mean white blood cell count, absolute lymphocytes and basophils in the females; an increase in mean percentage of large unclassified cells in males and females; and an increase in mean corpuscular hemoglobin in the females. All of these differences were within the expected range of normal and were not considered clinically significant. Increases observed in mean prothrombin time and activated partial thromboplastin time (APTT) in the male recovery animals and in mean APTT of the female recovery animals were attributed to variations in bleeding technique. Serum chemistry values were unremarkable in the treated animals at termination of the treatment period. Following recovery there were a number of small, but statistically significant differences observed in serum chemistry parameters of the satellite animals. These included a decrease in mean blood urea nitrogen (males), sodium (males) and chloride (females) and increases in phosphorus and billirubin (males) and calcium, total billirubin and triglycerides (femalesj. All of these findings were within the range of normal values and were comparable to control following the termination of dosing. These findings were not considered clinically significant or related to treatment. One female (300 mgikg) which died on Day 19 exhibited an enlarged liver, ascites in the abdominal cavity and a reddened jejunum. This death was attributed to the wrapping procedure. There were no gross postmortem observations or alterations in organ weights that were attributed to treatment with the test material. Slight alterations were noted in several organ weights at termination of dosing or recovery. These included a statistically significant increase in absolute and relative liver weight in the 100 mg/kg females and statistically significant decreases in relative brain and ovary weights in the 1000mg/kg females at termination of recovery. These findings did not correlate with any histopathological findings and were not attributed to treatment. There were no test material related microscopic findings noted in any group. One male and one female (1000 mgikg) has epidermal acanthosis/hyperkeratosis. The

	male also exhibited slight focal epithelial spongiosis. Similar lesions were observed in the skin of one untreated control female. These findings were attributed to the repeated clipping and tape irritation in both treated and sham control animals. No skin changes were noted following recovery. Livers from rats of all groups (including control) sacrificed after 28 days of treatment exhibited focal or multifocal necrosis. This was an acute change which was characterized by coagulative necrosis of hepatocytes and occurred in a random fashion. This finding has been seen in other dermal studies and has been attributed to trauma and/or ischemia to the liver resulting from the wrapping and manipulation of the animals. Liver necrosis was not evident in any of the satellite recovery animals.
Conclusions	A NOAEL of 1000 mg/kg was established for this study. Dermal application of this test material resulted in no signs of overt toxicity.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/15/00 (RTA-009)

5.3 Genetic Toxicity:

Robust Summary 3-Gentox-1

Test Substance	
CAS #	Analog of CAS# 70024-69-o
Chemical Name	C20-24 alkaryl calcium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1989
Species	Mouse
Strain	Swiss Albino Crl: CD-I (ICR) BR 50 days of age at initiation of treatment
Route of administration	Intraperitoneal
Duration of test	Single dose followed by 72-hour evaluation period.
Doses/concentration levels	0, 100, 200,400 and 500 mg/kg
Dose volume	**5** ml/kg
scx	Males and females
Frequency of treatment	Once
Control and treatment groups	Peanut oil vehicle control: 1 X/sex; triethylenemelamine positive control: 0.25 mglkg, 5/sex; 100 and 500 mglkg: 1 5/sex; 200 and 400 mglkg: 18/sex
Statistical methods	Animal to animal variability in spontaneous frequency of micronucleated polychromatic erythrocytes were evaluated in vehicle controls. Statistically significant differences were evaluated in the frequency of micronucleated polychromatic erythrocytes between treated groups and vehicle controls. NCE/PCE (normochromatic erythrocytes/polychromatic erythrocytes) ratios in treated and control groups were compared. Tests included dispersion test of Amphlett and Delow, and Margolin, Fishers exact test, binomial approximation, Cochran-Armitage test for trend, a one-way analysis of variance and Dunnett's procedure.
Dose rangefinding study	A rangefinding study was conducted at 200,400 and 600 mglkg. Mortality and physical observations were evaluated.
Remarks field for test conditions	All animals were observed frequently for physiological or behavioral abnormalities on the day of dosing and at least twice daily thereafter. Body weights taken on first day of the study prior to treatment and at sacrifice. Macroscopic pathology performed on all animals at sacrifice. Five/sex from each treatment group and vehicle control group were sacrificed for bone marrow sampling 24, 48 and 72 hours post treatment. Positive controls sampled at 24 hours only. NCE/PCE ratio and %PCE of total erythrocytes were calculated by counting a total of ≥1000 erythrocytes/animal. A total of 1000 PCE /animal were evaluated for the presence of micronuclei. (Guideline calls for 2000/animal to be evaluated.) The number of micronuclei in NCEs was also determined.

Results	
Remarks	During the dose rangefinding study mortality (9 of 10 animals) was observed at 600 mg/kg but not at lower dose levels. Signs of toxicity observed at all dose levels included reduced feces, reduced food consumption, hyperactivity and phonation. Decreased motor activity was observed at 400 and 600 mg/kg. Based on these results dose levels of 100, 200, 400 and 500 mg/kg were selected for the main study. During the main study toxicity was observed at 400 and 500 mg/kg. At 500 mg/kg 5 males and 4 females of 15/sex died prior to the scheduled sampling time. At 400 mg/kg 1 of 18 treated females died on Day 3. Other clinical signs of toxicity included palpebral closure, decreased motor activity and weakness. Cytotoxicity was observed in both sexes. A statistically significant increase in NCE/PCE ratio was observed in males at 500 mg/kg at 24 hours. Elevated ratios were also observed in individual animals of both sexes in other groups. Altered proportions of erythrocytes to nucleated cells were noted for both sexes in the treated groups. No biological or statistical significant increase in the number of micronucleated-PCE was observed in any treated group compared to the vehicle control. All values for individual animals were within the expected range of micronucleated-PCE/1 000 PCE expected for control animals. The variability in response observed in the treated animals was similar to that observed in the vehicle controls. The positive control exhibited a statistically significant increase in micronuclei as expected. Chemical analysis confirmed that the dosing solution preparation procedure utilized for this study resulted in homogeneous solutions of appropriate concentration.
Conclusions	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes of mice. The genotoxicity NOEL was 500 mg/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2110100 (RTA-005)

Robust Summary 3-Gentox:-2

Test Substance	
CAS #	CAS# 68783-96-O
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1995
Species	Mouse
Strain	Swiss Albino CD- 1; 10- 12 weeks of age at initiation of dosing
Route of administration	Oral gavage
Duration of test	Three treatment days followed by a 24-hour holding period.
Doses/concentration levels	0, 500, 1000, 2000 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Three treatments administered approximately 24 hours apart.
Control and treatment groups	Peanut oil vehicle control: 5/sex; Cyclophosphamide positive control: 20 mgikg (in water), 5/sex; 500, 1000 and 2000 mg/kg: S/sex
Statistical methods	Data summarized by sex and dose group/time point. Analysis performed using an analysis of variance, Dunnett's test, Cochran-Armitage test for linear trend Wilk's Criterion or Kolomogorov-Smirnov statistic, Kruskal-Wallis, Dunn's Summed Rank Test and Jonkheere's test of ordered response.
Dose Rangefinding Studies	Doses: 0.5, 1.O and 2.0 g/kg; 2/sex/dose sacrificed 24 hours after dosing. Percent polychromatic erythrocytes (PCE) was determined by counting 1000 cells. Number of micronuclei/l000 PCE determined.
Remarks field for test conditions	All animals were observed after dosing for signs of toxicity. Animals were examined twice daily for viability. Body weights were recorded prior to initiation of dosing. The animals from each group were sacrificed for bone marrow sampling 24 hours after the third dose. Necropsies were not performed. 2000 PCEs from each animal were examined for the presence of micronuclei. The percent of PCE in the total population of erytbrocytes was determined for each animal by counting a total of 1000 polychromatic and normochromatic erythrocytes. If the test article induced neither a statistically significant dose response nor a statistically significant increase at any dose level above concurrent vehicle, at any sampling point, it was considered negative.
Results	
Remarks	All dose rangefinding animals survived and were free of clinical signs. Bone marrow toxicity was not observed at any dose levels tested. Therefore 2000

	mg/kg was selected as the high dose for the micronucleus assay. The mid and low doses were selected to be 1/2 and 1/4 of the high dose. In the main study, all vehicle, positive control and treated animals were normal after dosing and remained healthy until sacrifice. There were no dose related increases or statistical differences in micronuclei formation observed at any dose level. Cytotoxicity was not observed since there were no atatistically significant decreases in the percentage of polychromatioc erythrocytes compared to the vehicle control. The positive control induced a statistically significant increase in mean micronucleated PCEs in both sexes compared to the vehicle controls which indicated the positive control was clastogenic and responded appropriately. The positive control also induced cytotoxicity. Chemical analysis confirmed the uniformity and stability of the test material in peanut oil for at least 9 days at all three concentrations. Concentration verification analysis confirmed that each dose level was within 3% of nominal concentration.
Conclusions	The test material was not genotoxic under the conditions of this study. The genotoxicity NOEL was 2000 mg/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/18/00 (RTA-022)

Robust Summary 3-Gentox: -3

Test Substance	
CAS #	CAS# 68783-96-O
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1984
Test System	L5178Y-3.7.2C mouse lymphoma cells
Culture Preparation and Maintenance	Cells were stored frozen in liquid nitrogen. Cultures were incubated at 37°C with shaking. Cultures were diluted daily to a cell density of approximately 3 x 10^5 cells/ml. Cultures were checked for bacterial and fungal contamination. Prior to use cultures were treated with methotrexate to reduce the frequency of spontaneously occurring TK-/- cells.
Exposure Method	Dilution
Test Substance Dosesiconcentration levels	Concentrations of 500, 1000, 1500, 2000, 4000 and 5000 ug/mL were evaluated with and without metabolic activation.
Metabolic Activation	Aroclor induced rat liver
Vehicle	Dimethyl sulfoxide (DMSO) 10 ul/mL
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 744 ug/mL
Statistical Analysis	Means and standard deviations were determined.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test.
Dose range finding study	Test substance (dose levels from 1 to 10,000 ug/mL) and vehicle control tested with and without activation. Cultures were exposed to the test substance and incubated for approximately four hours, then washed and cultured for two days. Cell culture density was determined 24 and 48 hours post exposure. Treated cell suspension growth at each dose level was compared to the negative solvent control.
Remarks field for test conditions	Prior to study initiation the solubility of the test substance and of the positive control materials in the vehicle (DMSO) was confirmed. A pretest dose range finding study was conducted at concentrations up to 10,000 ug/mL with and without metabolic activation. In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation. The test material was prepared so that the highest and lowest concentrations would yield percent total growth of approximately 10% and 90% respectfully. The test material was added to cells with and without

	activation and incubated for four hours. Cells were then washed and placed in suspension cultures for two days with a cell population adjustment at 24 hours. The cells were then plated in a restrictive media containing trifluorothymidine (TFT) which allows TK+/- cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability. Plates were incubated at 37°C in a humidified 5% CO_2 atmosphere for IO- 12 days. Following incubation all plates were scored for total number of colonies/plate. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity plates to the average number of colonies in the corresponding viability plates. For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 0.2 to 2.0/1 0^4 cells; negative control plating efficiency should be at or above 50% and the test material should be tested to the level of approximately 10% total growth or to the limits of solubility or to a high dose of 100 mg/mL.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	The dose rangefinding study indicated significant toxicity (<90% total growth) at 500 ug/mL with and without metabolic activation. Based on these results the test material was evaluated for mutagenicity at concentrations ranging from 500 to 5000 ug/mL. Six cultures with and without activation were selected for cloning at 500, 1000, 1500, 2000, 4000 and 5000 ug/mL. None of the cultures treated with test material with or without activation exhibited mutant frequencies significantly different from the average mutant frequency of the negative (solvent) controls at a percent total growth of 10% or greater. Positive and vehicle control group responses were appropriate and met the criteria outlined above.
Conclusions	The test material was not genotoxic under the conditions of this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 4/11/00 (RTA-028)

Robust Summary 3-Gentox-4

Test Substance	
CAS #	CAS# 68783-96-O
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 47 1
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1995
Test System	*Salmonella typhimurium*
Strains Tested	TA98, TAIOO, TA1535, TA1537, TA1538
Exposure Method	Plate incorporation
Test Substance Dosesiconcentration levels	250, 500, 1000, 2500 and 5000 ug/plate (initial assay) 1000, 2000, 3000, 4000 and 5000 ug/plate (repeat assay)
Metabolic Activation	With and without (0.5 mL S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats. –S9 groups received 0.5mL saline).
Vehicles	Tetrahydrofuran (THF, for test material), Dimethylsulfoxide (DMSO, for positive control substances)
Positive Controls and concentration levels by tester strain and activation status	9-Aminoacridine (9AA), 100 ug/plate- TA 1537 without S9 2-Aminoanthracine (2AA), 2.5 ug/plate-all strains with S9 N-Methyl-N-NitroN-Nitrosoguanidine (MNNG), IO ug/plate-TA100, TA1535 without S9 2-Nitrofluorene (2NF), 5 ug/plate-TA98, TA1538 without S9
Vehicle Controls	Tetrahydrofuran 25 uL/plate Dimethylsulfoxide 100 uL/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Range finding Study	Conducted using tester strain TAIOO at concentrations up to 5000 ug/plate with and without metabolic activation. Cytotoxicity was evaluated.
Remarks field for test conditions	This study was conducted according to OECD Guideline 471 (1983). Revision to this Guideline in 1997 suggests the addition of the *E. coli* WP2 uvrA or *S. typhimurium* TA 102 tester strains. Since this study was conducted prior to this revision, these strains were not included. Prior to study initiation the solubility of the test substance in the vehicle (tetrahydrofuran) was confirmed. A pretest dose range finding study was conducted using tester strain TAIOO at concentrations up to 5000 ug/plate with and without metabolic activation. In the main study there were two treatment sets for each tester strain, with and without metabolic activation. Each of the five tester strains was dosed with five concentrations of test substance (250, 500, 1000, 2500 and 5000 ug/plate), two vehicle controls (THF and DMSO), a nontreated control and a positive control. Three plates/dose

	group/strain/treatment set were evaluated. The results of the initial assay were verified by repeating the assay at dose levels of 1000, 2000, 3000, 4000 and 5000 ug/plate. After 2 days of incubation all plates in the initial and repeat assays were evaluated for gross toxic effects and total revertant colony numbers.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	Toxicity (notable reduction in background lawn and/or 50% reduction in the number of revertant colonies compared to vehicle control) was not observed at any concentration tested with or without metabolic activation in the range finding study. However at the 5000 and 2000 ug/plate levels a haze attributed to the test substance was present. These findings resulted in the selection of concentrations of 250, 500, 1000, 2500 and 5000 ug/plate for the initial study. The test substance did not induce significant increases in revertant colonies (equal to or greater than three times the THF control) in any of the tester strains, at any dose level, with or without metabolic activation in the initial or repeat assays. Beading of the test substance was observed at 5000 ug/plate in all tester strains (with/without activation) and at 4000 ug/plate in tester strain TA1537 (with/without activation) in the repeat assay. The positive controls produced at least a three-fold increase in revertant colonies when compared with the DMSO control in each respective strain. The nontreated and vehicle controls responded appropriately. The 5000 ug/plate concentration of test substance in THF was evaluated analytically for concentration in both the initial and repeat assays. Analysis conformed that the test substance concentration was within 7% of the nominal concentration for both assays.
Conclusions	The test substance was not mutagenic in any strain of *Salmonella typhimurium* tested, including at least one dose above the solubility of the test substance. The genotoxicity NOEL was 5000 ug/plate.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/18/00 (RTA-021)

Robust Summary 3-Gentox--5

Test Substance	
CAS #	CAS# 7 1786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 473
Test Type	*In Vitro* Chromosomal Aberration Assay in CHO Cells
GLP (Y/N)	Y
Year (Study Performed)	1995
Test System	Chinese hamster ovary cells
Clone Tested	WBL
Culture Preparation and Maintenance	Cells were thawed and cultured in McCoy's 5A Medium containing 10% fetal bovine serum and 2 mM L-glutamine at 37°C, in 4-6% CO_2 in air, Cultures were seeded at 1.2 x 10" cells (16-hour harvest) and 0.8 x 1 0^6 (40-hour harvest) approximately 1 day prior to dosing. Fetal bovine serum was excluded from activated cultures.
Exposure Method	Dilution
Test Substance Doses/concentration levels	A 50 uL sample of concentrations of 10, 20, 40, 80, 120, 160 ug/mL was evaluated with and without metabolic activation.
Metabolic Activation	With and without (0.015 mL/ mL serum free medium) S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats and 0.06 mL/ mL serum free medium cofactor mix (13.4 mg/mL NADP and 25 mg/mL DL-Isocitric Acid in distilled water).
Vehicles	Tetrahydrofuran (THF, for test material), acetone (for positive control substances)
Vehicle and Positive Control concentration levels by activation status	Acetone, 5 ug/mL with and without activation Tetrahydrofuran, 5 ug/mL with and without activation N-Methyl-N-Nitro-N-Nitrosoguanidine (MNNG), 0.6 ug/mL without activation 7,12-Dimethylbenz[a]anthracene (DMBA), 10 ug/mL with activation
Statistical Analysis	The number of cells with at least one aberrant chromosome and the number of cells examined in each replicate were used for statistical analysis. The number of aberrant individual chromosomes/cell was not analyzed. Positive control groups were compared to vehicle control by Fisher Exact Test. Each pair of replicates was compared by Fisher Exact Test. Differences between control and treated groups were compared using Fisher Exact Test and if necessary a 2x2 Fisher Tests. A permutation test was performed to test for dose related trends. Significance levels of less than 0.05 were reported.
Test Substance Solubility	Test substance solubility in the vehicle was determined.
Culture Medium Solubility Test	The solubility of the test substance in the culture medium was established at concentrations of 10, 20, 39, 78, 156, 3 13, 625, 1250, and 2500 ug/mL. Visual and microscopic examinations were made for precipitation at 0, 30 and 180 minutes post preparation. Concentrations showing signs of insolubility at any

	of these time points were considered unsuitable for dosing.
Dose range finding study	Test substance and vehicle controls tested in duplicate cultures each with and without activation. Test substance tested at concentrations of 2.5, 5, 10, 20, 40, 60, 80, 120, and 160 ug/ml. Cytotoxicity and mitotic indices were evaluated.
Remarks field for test conditions	Prior to study initiation the solubility of the test substance and of the positive control materials in the vehicles (tetrahydrofuran/acetone) was confirmed. A pretest dose range finding study was conducted at concentrations up to 160 ug/mL with and without metabolic activation. In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and MNNG (positive control) was tested without activation. Prepared cultures were treated with test substance or control material and were incubated for 16 hours. A repeat assay was performed using 16 and 40 hour harvest time points. Vehicle, MNNG and DMBA cultures were incubated for 16 hours only. Two to three hours prior to the 16 and 40 hour harvest the spindle inhibitor, Colcemid, was added to each culture to obtain a final concentration of 0.2 ug/mL. Harvested cells were evaluated microscopically for percent confluency, morphology and estimated number of mitotic cells prior to harvest. The test substance treated groups, selected for chromosome analysis based on cell count data and the presence of participate, were as follows: Initial assay +S9 (16 hour harvest) 20, 40 80 ug/mL Initial assay -S9 (16 hour harvest) 20, 40 80 ug/mL Repeat assay +S9 (16 hour harvest) 20, 40 80 ug/mL Repeat assay -S9 (16 hour harvest) 20, 40 80 ug/mL Repeat assay +S9 (40 hour harvest) 80, 120, 160 ug/mL Repeat assay -S9 (40 hour harvest) 80, 120, 160 ug/mL Slides were prepared for these groups using Giemsa stain. Two slides/treatment group were evaluated. 200 metaphase cells (100 per culture) each containing 19-23 chromosomes per treatment group were scored. Chromosomes were counted for each cell. Chromosome aberrations, either chromosome or chromatid type were recorded. The following observations were recorded and excluded from the total aberration frequency: gaps, polyploid and endoreduplicated cells, pulverized chromosomes, Robertsonian translocations, translocations and abnormal monocentric chromosomes. The percent of aberrant cells and the frequency of aberration (%) per treatment group were determined. In order for a test substance to be considered to have induced a positive response compared to vehicle control a statistically significant dose related increase in the percentage of aberrant cells along with a mean percentage of aberrant cells in excess of 5% in at least one treatment group were required. Or, a reproducible and statistically significant response in at least one treatment group with a mean % of aberrant cells exceeding 5% was observed. Test substance concentration verification was performed on the highest stock concentration in both the initial and repeated assays. Results were within 8% of nominal.

Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	In the culture medium solubility test precipitate and/or cloudiness were present with and without metabolic activation at concentrations of 39 ug/mL and 78 ug/mL and greater. In the pretest toxicity assay, a greater than 50% reduction in cell counts or mitotic activity was not observed at concentrations up to 160 ug/mL. The doses selected for the initial assay were 10, 20, 40, 80, 120 and 160 ug/mL. Cell survival was not significantly reduced when compared to the vehicle control in the initial assay. Cell survival was reduced by at least 50% compared to vehicle control in the repeat assay (40-hr harvest) without metabolic activation at the 160 ug/mL concentration. A greater than 50% reduction in mitotic index was not observed in either the initial or repeat assays at any concentration tested. Precipitation was observed at concentrations greater than 80 ug/mL in the chromosomal aberration assay. Therefore, the highest concentration evaluated at 16 hours was 80 ug/mL. There were no statistically significant differences in the number of chromosomal aberrations at 16 hours with activation and at 40 hours with and without metabolic activation. In the initial 16-hour harvest without activation a statistically significant increase was observed with one dose level different from the vehicle control. However this finding was not evident in the repeat 16-hour harvest without activation. The observed initial increase was not reproducible and was not considered biologically significant. Positive and vehicle control group responses were as expected. The positive control group had a statistically significant higher percentage of aberrant cells than the vehicle control group with and without activation at each harvest interval.
Conclusions	The test material was not genotoxic under the conditions of this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 4/10/00 (RTA-026)

Robust Summary 3-Gentox--6

Test Substance	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1995
Species	Mouse
Strain	CD-l, 10-12 weeks of age at initiation of treatment
Route of administration	Oral gavage
Duration of test	Three treatments administered approximately 24 hours apart followed by a 24-hour hold period prior to bone marrow sample collection.
Doses/concentration levels	0, 500, 1000 and 2000 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Three treatments administered approximately 24 hours apart.
Control and treatment groups	Peanut oil vehicle control: S/sex; cyclophosphamide (in water) positive control: 20 mg/kg, 5/sex; 500, 1000, 2000 mg/kg, S/sex/kg.
Statistical methods	The following parameters were recorded and evaluated; the ratio of polychromatic to normochromatic etythrocytes, number of polychromatic erythrocytes with micronuclei and number of polychromatic erythrocytes scored. Statistical analysis included means and standard deviations of the micronuclei data and a test of equality of group means. Tests included a one-way analysis of variance, Duncan's Multiple Range test and regression analysis. Residuals from the ANOVA were analyzed by Wilk's Criterion or the Kolomogorov-Smirnov statistic. Nonparametric analyses included the Kruskal-Wallis one way ANOVA followed by Dunn's Summed Rank Test. Dose response was evaluated by Jonkheere's test of ordered response.
Dose range finding study	A dose range finding study was conducted at 500, 1000 and 2000 mg/kg. Percent polychromatic erythrocytes (PCE) were determined by counting 1000 cells. Number of micronuclei/l000 PCE determined.
Remarks field for test conditions	All animals observed for viability twice daily during the dosing period. Detailed clinical observations recorded after each test substance administration. Body weights recorded prior to initiation of dosing. Twenty-four hours after the third dose the animals were sacrificed for bone marrow sampling. Necropsies were not performed. A total of 2000 polychromatic erythrocytes/animal were evaluated for the presence of micronuclei. The percent of PCE in the total population of erythrocytes was determined for each animal by counting the total polychromatic and normochromatic erythrocytes.

Results	
Remarks	All dose rangefinding animals survived and were free of clinical signs. Bone marrow toxicity was not observed at any dose levels tested. Therefore 2000 mg/kg was selected as the high dose for the micronucleus assay. The mid and low doses were selected to be 1/2 and 1/4 of the high dose. All animals survived to scheduled sacrifice and were free of clinical signs. The responses of the vehicle control and positive control groups were appropriate and support the validity of the assay results. The positive control induced a significant increase in mean number of micronucleated polychromatic erythrocytes. In addition it induced cytotoxicity. There were no dose-related increases or statistical differences in micronuclei formation observed at any dose level of the test material. Cytotoxicity was not observed. There were no statistical decreases in the percentage of polychromatic erythrocytes compared to the vehicle control. Chemical analysis of dos ing solutions confirmed that they were homogeneously prepared at the desired concentrations and that they were stable for the intended period of use.
Conclusions	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes and did not induce cytotoxicity in the bone marrow of CD- 1 mice. The genotoxicity NOEL was 2000 mg/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/11/00 (RTA-007)

Robust Summary 3-Gentox-7

Test Substance	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 47 1
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1995
Test System	*Salmonella typhimurium*
Strains Tested	TA98, TA100, TA1535, TAI 537, TA1538
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	62.5, 125, 250, 500 and 1000 ug/plate
Metabolic Activation	With and without (0.5 mL S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats. –S9 groups received 0.5mL saline.
Vehicles	Tetrahydrofuran (THF, for test material), Dimethvlsulfoxide (DMSO, for positive control substances)
Positive Controls and concentration levels by tester strain and activation status	9-Aminoacridine (9AA), 100 ug/plate- TA1537 without S9 2-Aminoanthracine (2AA), 2.5 ug/plate-all strains with S9 N-Methyl-N-Nitro-N-Nitrosoguanidine (MNNG), 10 ug/plate-TA100, TA1535 without S9 2-Nitrofluorene (2NF), 5 ug/plate-TA98, TA1538 without S9
Vehicle Controls	Tetrahydrofuran 25 uL/plate Dimethylsulfoxide 100 uL/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose rangefinding study	Conducted using tester strain TA100 at concentrations up to 5000 ug/plate with and without metabolic activation.
Remarks field for test conditions	This study was conducted according to OECD Guideline 47 1 (1983). Revision to this Guideline in 1997 suggests the addition of the *E. coli* WP2 uvrA or S. *typhimurium* TA 102 tester strains. Since this study was conducted prior to this revision, these strains were not included. Prior to study initiation the solubility of the test substance in the vehicle (tetrahydrofuran) was confirmed. A pretest dose range finding study was conducted using tester strain TA100 at concentrations up to 5000 ug/plate with and without metabolic activation. In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the five tester strains was dosed with five concentrations of test substance, two vehicle controls (THF and DMSO), a nontreated control and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were verified by repeating the assay. After 2

	days of incubation all plates in the initial assay and the TA1537 and TA1538 plates in the repeat assay were refrigerated. These plates were evaluated for gross toxic effects and total revertant colony numbers on the following day. In the repeat assay TA98, TA100 and TA1535 were evaluated after 2 days of incubation.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	In the range finding study toxicity (notable reduction in background lawn and/or 50% reduction in the number of revertant colonies compared to vehicle control) was not observed at any concentration tested with or without metabolic activation. However the 5000 and 2000 ug/plate levels were difficult to evaluate due to test substance interference. At 1000 and 500 ug/plate precipitate was observed on the plates. These findings resulted in the selection of concentrations ranging from 62.5 to 1000 ug/plate for the main study. The test substance did not induce significant increases in revertant colonies (equal to or greater than three times the THF control) in any of the tester strains, at any dose level, with or without metabolic activation in the initial or repeat assays. A greater than 50% reduction in mean number of revertant colonies compared to THF were observed in the initial assay in TA1537 without activation at 2.50 ug/plate. In TA1535 with activation, no background /no revertants was noted in the initial assay for all three plates at 250 and 500 and in two plates at 1000 ugiplate. The significance of these reductions is difficult to interpret since the findings were inconsistent between assays and dose levels. Precipitate was seen on all plates at 1000 ug/plate (+/- S9) in the initial and repeat assays. The positive controls produced at least a three-fold increase in revertant colonies compared with the DMSO control in their respective strains. Nontreated and vehicle controls were acceptable and were consistent with data from previous assays. The 1000 ugiplate concentration of test substance in THF was evaluated analytically for stability, concentration and homogeneity. Analysis conformed **that** the test substance was stable and homogeneous in THF for the intended period of **use.** The 1000 ug/plate solution was prepared and assayed twice during the study. The result for the first preparation was 12 1% above nominal. The result of the second preparation was 15% above nominal.
Conclusions	The test substance was not mutagenic in any strain *of Salmonella typhimurium* tested, including at least one dose above the solubility of the test substance. The genotoxicity NOEL was 1000 ug/plate.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/1 4/00 (RTA-008)

Robust Summary 3-Gentox-8

Test Substance	
CAS #	CAS# Analog of 78330-I 2-8
Chemical Name	C 15-C2 1 alkaryl sodium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline Followed	OECD Guideline 47 1
rest Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	*Salmonella typhimurium*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA 1538
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0.1, 0.3, 1.0, 3.0 and 10 mg/plate
Metabolic Activation	With and without 25 ul/plate S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Sterile distilled water
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 2-aminoanthracene 2.0 ug/plate TA98 -S9 2-nitroflourene 10.0 ug/plate TA100 +S9 2-aminoanthracene 2.0 ug/plate TA100 -S9 sodium azide 1.O ug/plate TAI 535 +S9 2-aminoanthracene 2.0 ug/plate TA1535 -S9 sodium azide 1.Oug/plate TA1537 +S9 2-aminoanthracene 2.0 ug/plate TA1537 -S9 9-Aminoacridine 50.0 ug/plate TA1538 +S9 2-aminoanthracene 2.0 ug/plate TA1538 -S9 2-nitroflourene 10.0 ug/plate
Vehicle Control	Sterile distilled water
statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Range finding Study	Conducted using tester strain TA1 00 at dose levels of test material ranging from 0.005 to 10 mg/plate without S9.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a dose level of test material of 10 mg/plate and concentrations of S9 mix ranging from 25 to 250 ul S-9/plate.
Remarks field for test conditions	This study was conducted according to OECD Guideline 471 (1983). Revisions to this Guideline in 1997 suggest the addition of the *E. coli* WP2 uvrA or *S. typhimurium* TA 102 tester strains. Since this study was conducted prior to this revision, these strains were not included. In the main study there were two treatment sets for each tester strain, with (+S9)

	and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. 0.1 ml of test material, positive control or vehicle control were added to each plate along with 0.1 ml of tester strain, S9 mix (if needed) and 2.0 ml of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	Slight cytotoxicity was observed in the dose range finding study with tester strain TA 100 without metabolic activation. The S9 optimization study was performed using TA98 and TA 100 at 10 mg/plate and concentrations of S9 mix of 25-250 ul. In the absence of any effect 25 ul S9 mix/plate was used in the mutagenicity study. In the main study the test material was not mutagenic to any strain. It was slightly cytotoxic to TA100 in the absence of metabolic activation. Positive control responses were acceptable.
Conclusions	Under the conditions of this study, the test material was not mutagenic with or without metabolic activation.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 10/4/00 (RTA-070)

Robust Summary 3-Gentox-9

Test Substance	
CAS #	Analog of CAS# 70034-69-0
Chemical Name	C20-C24 alkarvl calcium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 47 1
Test **Type**	Bacterial Reverse Mutation Assay
GLP (Y/N)	v
Year (Studv Performed)	1989
Test System	*Salmonella typhimurium* and *Escherichia Coli*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1.535, TA1.537; *Escherichia Coli* tester strain WP2*uvr*A
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0.1. 0.33, 1.0, 3.33 and 10 mg/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Pluronic F 127 25% w/w in ethanol
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 2-aminoanthracene 2.0 ug/plate TA98 -S9 2-nitroflourene 10.0 u&late TAlOO +S9 2-aminoanthracene 2.0 ug/plate TA 100 -S9 sodium azide 1.0 ug/plate TA1535 +S9 2-aminoanthracene 2.0 ug/plate TA1535 -S9 sodium azide 1.0 ug/plate TA1537 +S9 2-aminoanthracene 2.0 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2*uvr*A +S9 2-aminoanthracene 80.0 ug/plate WP2*uvr*A –S9 ICR-191 50.0 ug/plate
Vehicle Control	Pluronic F127 25% w/w in ethanol
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA98 and TA100, and dose levels of test material ranging from 0.003 to 10 mg/plate.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a dose level of test material of 10 mg/plate and concentrations of S9 mix ranging from 25 to 400 ul S-9/plate. Cytotoxicity was evaluated.
Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial

	assay were confirmed in a second independent experiment. 100 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 ml of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	**No** cytotoxicity was observed in the dose rangefinding study with tester strains TA100 and WP2*uvr*A with or without metabolic activation as evidenced by normal background lawn and no reduction in the number of revertants/plate. The S9 optimization study was performed using TA98 and TA100 with the highest non-cytotoxic dose of test article, (10,000 ug/plate) and concentrations of S9 mix of 25-400 ul. In the absence of any effect 25 ul S9 mix/plate was used in the mutagenicity study. The test material formed a stable emulsion with the vehicle and the dilutions were well dispersed in the top agar. However after incubation test material was visible at all dose levels in the top layer. The test material was not cytotoxic to any tester strain. In the repeat study statistically significant increases in revertant colonies were observed in TA1535 without metabolic activation and in WP2*uvr*A with metabolic activation. However since these findings were not Found during the first experiment they were not considered biologically significant. The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response. Dosing solution analysis confirmed that high dose concentration was acceptable.
Conclusions	Under the conditions of this study, the test material was not mutagenic with or without metabolic activation.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 10/4/00 (RTA-069)

Robust Summary 3-Gentox10

Test Substance	
CAS #	CAS# 68783-96-O
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 473
Test Type	*In Vitro* Chromosomal Aberration Assay in CHO Cells
GLP (Y/N)	Y
Year (Study Performed)	1995
Test System	Chinese hamster ovary cells
Clone Tested	WBL
Culture Preparation and Maintenance	Cells were thawed and cultured in McCoy's 5A Medium containing 10% fetal bovine serum and 2 mM L-glutamine at 37°C, in 46% CO_2 in air. Cultures were seeded at 1.2 x 10" cells (16-hour harvest) and 0.8 x 10^6 (40-hour harvest) approximately 1 day prior to dosing. Fetal bovine serum was excluded from activated cultures.
Exposure Method	Dilution
Test Substance Doses/concentration levels	A 50 uL sample of concentrations of 10, 20, 40, 80, 120, 160 ug/mL was evaluated with and without metabolic activation.
Metabolic Activation	With and without (0.015 mL/ mL serum free medium) S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats and 0.06 mL/ mL serum free medium cofactor mix (13.4 mg/mL NADP and 25 mg/mL DL-lsocittic Acid in distilled water).
Vehicles	Tetrahydrofuran (THF, for test material), acetone (for positive control substances)
Vehicle and Positive Control concentration levels by activation status	Acetone, 5 ug/mL with and without activation Tetrahydrofuran, 5 ug/mL with and without activation N-Methyl-N-NitroN-Nitrosoguanidine (MNNG), 0.6 ug/mL without activation 7,12-Dimethylbenz[a]anthracene (DMBA), 10 ug/mL with activation
Statistical Analysis	The number of cells with at least one aberrant chromosome and the number of cells examined in each replicate were used for statistical analysis. The number of aberrant individual chromosomes/cell was not analyzed. Positive control groups were compared to vehicle control by Fisher Exact Test. Each pair of replicates was compared by Fisher Exact Test. Differences between control and treated groups were compared using Fisher Exact Test and if necessary a 2x2 Fisher Tests. A permutation test was performed to test for dose related trends. Significance levels of less than 0.05 were reported.
Test Substance Solubility	Test substance solubility in the vehicle was determined.
Culture Medium Solubility Test	The solubility of the test substance in the culture medium was established at concentrations of 10, 20, 39, 78, 156, 313, 625, 1250, and 2500 ug/mL. Visual and microscopic examinations were made for precipitation at 0, 30 and 180 minutes post preparation. Concentrations showing signs of insolubility at any

	of these time points were considered unsuitable for dosing.
Dose rangefinding study	Test substance and vehicle controls tested in duplicate cultures each with and without activation. Test substance tested at concentrations of 0.625, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 ug/ml. Cytotoxicity and mitotic indices were evaluated.
Remarks field for test conditions	Prior to study initiation the solubility of the test substance and of the positive control materials in the vehicles (tetrahydrofuran/acetone) was confirmed. A pretest dose range finding study was conducted at concentrations up to 160 ug/mL with and without metabolic activation. In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and MNNG (positive control) was tested without activation. Prepared cultures were treated with test substance or control material and were incubated for 16 hours. A repeat assay was performed using 16 and 40 hour harvest time points. Vehicle, MNNG and DMBA cultures were incubated for 16 hours only. Two to three hours prior to the 16 and 40-hour harvest the spindle inhibitor, Colcemid, was added to each culture to obtain a final concentration of 0.2 ug/mL. Harvested cells were evaluated microscopically for percent confluency, morphology and estimated number of mitotic cells prior to harvest. The test substance treated groups, selected for chromosome analysis based on cell count data and the presence of participate, were as follows: Initial assay +S9 (16 hour harvest) 10, 20, 40 ug/mL Initial assay -S9 (16 hour harvest) 10, 20, 40 ug/mL Repeat assay +S9 (16 hour harvest) 10, 20, 40 ug/mL Repeat assay -S9 (16 hour harvest) 10, 20, 40 ug/mL Repeat assay +S9 (40 hour harvest) 10, 20, 40 ug/mL Repeat assay -S9 (40 hour harvest) 10, 20, 40 ug/mL Slides were prepared for these groups using Giemsa stain. Two slides/treatment group were evaluated. 200 metaphase cells (100 per culture) each containing 19-23 chromosomes per treatment group were scored. Chromosomes were counted for each cell. Chromosome aberrations, either chromosome or chromatid type were recorded. The following observations were recorded and excluded from the total aberration frequency: gaps, polyploid and endoreduplicated cells, pulverized chromosomes, Robertsonian translocations, translocations and abnormal monocentric chromosomes. The percent of aberrant cells and the frequency of aberration (%) per treatment group were determined. In order for a test substance to be considered to have induced a positive response compared to vehicle control a statistically significant dose related increase in the percentage of aberrant cells along with a mean percentage of aberrant cells in excess of 5% in at least one treatment group were required. Or, a reproducible and statistically significant response in at least one treatment group with a mean % of aberrant cells exceeding 5% was observed. Test substance concentration verification, uniformity and stability were nerformed on the highest stock concentration in both the initial and/or repeated

	assays. Results were within 6% of nominal. Samples were homogeneous and stable for the intended period of use.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	In the culture medium solubility test precipitate and/or cloudiness were present with and without metabolic activation at concentrations of 39 ug/mL and greater. In the pretest toxicity assay there was an 8 1% reduction (compared to vehicle control) in cell survival at 160 ug/mL without metabolic activation. The doses selected for the initial assay were 10, 20, 40, 80, 120 and 160 ug/mL. A greater than 50% reduction in cell survival and/or mitotic index was not observed in either the initial or repeat assays. Precipitation was observed at concentrations greater than 40 ug/mL in the chromosomal aberration assay. Therefore, 40 ug/mL was considered to be the limit of solubility for the test substance and was selected as the highest test concentration to be evaluated. There were no statistically significant differences in the number of chromosomal aberrations between the treated and vehicle control groups in either the initial or repeat assay at any dose level evaluated (10, 20 and 40 ug/mL with and without metabolic activation). In the initial 16-hour harvest, there were statistically significant increases with dose in the percent of aberrant cells for both the activated and nonactivated evaluations. These trends were not reproducible in the repeat 16-hour harvest and therefore were not considered biologically significant. Positive and vehicle control group responses were as expected. The positive control groups have frequencies of aberrations outside the normal range of the vehicle control and at least twice the vehicle control value.
Conclusions	The test material was not genotoxic under the conditions of this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 4111100 (RTA-027)